THE FIXATION OF AVIAN CHROMOSOMES

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FOUR FIGURES

When a successful preservation for cytological study of mammalian tissue had been accomplished (Hance, '17), the hope arose that the other warm-blooded group—the birds—might yield to the same treatment. The receipt of some beautifully fixed preparations of chick tissue cultures from Mrs. M. R. Lewis gave further expectation that equal results might be achieved with larger pieces of tissue put through the section method. Fortunately, the same methods that gave sharply defined chromosomes in mammalian cells gave comparable pictures with bird tissues (figs. 1 to 4).

The following technique was used first with chick embryos and later with the testes of adult roosters, pigeons, a robin, and sparrows.

Killing solution. Flemming's strong solution plus about ½ per cent urea and used at approximately 0°C. (solution kept on ice) was pipetted over the embryo as it lay in place on the yolk or very small pieces of testes were placed directly into the cold solution.

Time. In about a minute the embryo hardens sufficiently for removal to a vial containing the cold fixing solution. It is allowed to stay in this mixture for from two to twenty-four hours—three to four hours being ample for small embryos or pieces of tissue of such size as to come within the limited penetrating possibilities of osmic acid.

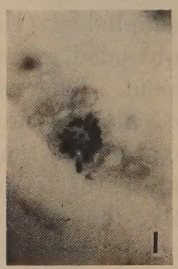
Washing. The tissues are washed in running water or in several changes of water for twelve to twenty-four hours.

THE ANATOMICAL RECORD, VOL. 31, NO. 2
OCTOBER, 1925

Dehydration. Drop method used (Allen, '16; Hance, '25; Painter, '24). This tissue is carried up to 95 per cent alcohol.

Clearing. Cedar oil is added to the tissue in 95 per cent alcohol. After thirty minutes to one hour it is placed in pure cedar oil. The oil is replaced with fresh cedar oil in about an hour and allowed to remain on the tissues for two hours or longer. The cedar oil is removed with chloroform.

Infiltrating medium. Chloroform and paraffin followed by pure paraffin. It takes about four hours to infiltrate small pieces of tissue with paraffin sufficiently hard (about 58° to 60°) to allow sectioning at 5 μ.

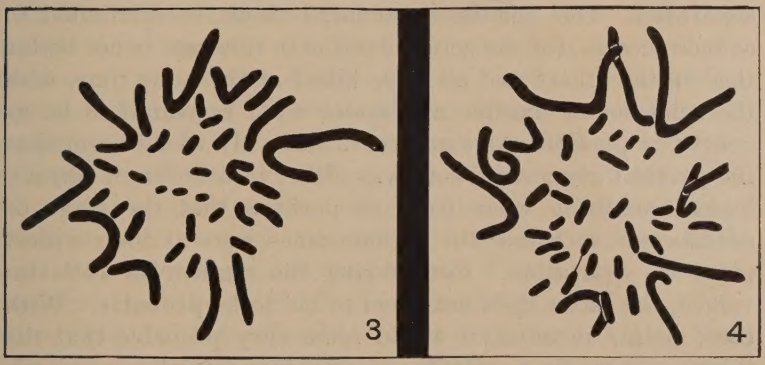


Figs. 1 and 2 Photomicrographs of dividing cells in chick embryos. Cold Flemming fixation.

With adult testes as good results have been obtained with Flemming's solution without the addition of urea. Urea was originally added to fixing fluids to increase the speed of penetration. Tissue cultures were fixed in Zenker, Zenker-acetic, and in osmic-acid vapor.

One hundred fifty-seven embryos, embryonic gonads and membranes and testes have been prepared by this method with successful results from a cytological standpoint. Thirty testes obtained when the fowl were caponized at about six weeks of age were mitotically inactive, and since such dividing cells as were found were not in the best state of preservation it was thought that the method that worked with the embryos had failed to give comparable results with the adult tissues. Consequently, a number of fixation tests (forty-eight in all)

were made, using the testes of pigeons to see if the preservation could be bettered. These tests in the end led back to the original method outlined above. Good results can be obtained with Flemming's solution used at room temperature, as is shown on a slide of pigeon testes in Doctor McClung's collection, fixed twenty-five years ago. This testis was in a very active state of division and well-preserved spermatogonial metaphases were found in the outermost side of the tubules that first came in contact with the killing solution. Dividing cells separated from the fluid by only a few cell layers were poorly fixed. The low temperature, then, seems to have some



Figs. 3 and 4 Camera-lucida drawings of chromosomes in chick embryo cells that have been fixed in cold Flemming solution.

influence on penetration or in holding the cellular processes in a life-like condition until the killing agent penetrates and fixes them. Good results were also obtained with a double fixation (no. 12) discussed below. Good histological fixation was obtained with practically all of the mixtures used, especially with the following groups numbered 2 and 6, but the chromosomes tended to clump together following these methods, and consequently were not good for cytological investigation.

Those familiar with the problems connected with the study of dividing cells know that the finding of cells preserved in workable condition is largely dependent upon the following conditions:

1. The cells of the tissues to be studied must be actively multiplying in order that among the many observed a few really good mitotic figures may be found. Since it is known that a technique that gives a maximum of good results does not preserve all cells equally, the laws of chance would operate against securing clear pictures in tissues possessing very few dividing cells.

2. The physiological condition may play a part in the results. In over thirty embryonic gonads studied more and better division figures were found in one of them than in the sum total of the others. The divisions in this gonad were not only more numerous, but the chromosomes were better separated. This condition cannot, I think, be attributed to chance success, for the actual fixation in this case is not better than in the others and all were killed at the same time, with the same lot of fixative and under what appeared to be as nearly as possible the same conditions. It would seem that the particularly good gonad was either in a different physiological condition when fixed or perhaps that the stage of mitosis was such that the chromosomes were at their widest point of separation. Considering the number of cells involved, the latter does not seem to me to be probable. With these points in mind, it would seem very probable that the thirty apparently poorly fixed samples referred to were adjudged poor because they did not happen to fulfill the requirements of nos. 1 and 2 above.

An indication of the combination of reagents used in the technique experiments in which the pigeon testes were used as material may be useful to other students. Formulae are omitted, but may be obtained from the writer. The following fluids were used at 0°C., room temperature, and at blood heat.

1. Flemming's solution with the acetic acid content varied widely.
2. Picro-formo-acetic with several variations in the proportion of acetic acid and formalin.
3. Chromic acid, formalin, and acetic acid.
4. Chrome-acetic.

5. Formalin, acetic acid, picric acid, chromic acid, and urea (Allen, '16).

6. The same as 5, with varying amounts of acetic acid and formalin and also with nitric acid in place of the acetic.

7. The same as 5, plus 0.01 per cent osmic acid. This small quantity of osmic acid has a recognizable effect and improves the fixation slightly.

8. Carnoy's solution with and without chloroform.

9. Tellyesnickey's fluid.

10. Carnoy-Lebrun.

11. Formalin, nitric acid, and mercuric bichloride. Also used with the addition of chromic acid and osmic acid.

12. The same as 8, but after immersion in these fluids for two or three minutes the tissue is placed in Flemming's fixative. This is the double fixation method with which Oguma and Kihara ('23) obtained very satisfactory results on human testes. The writers do not indicate whether Carnoy's fluid was used with or without chloroform and followed in two or three minutes Flemming's solution. Excellent preservation was obtained through the use of Carnoy's fluid containing chloroform followed by cold Flemming. The low temperature of the second fluid is a modification of the originator's method, but seemed to give better results. I am not entirely convinced that the double fixation is an improvement on the results obtained with cold Flemming alone, but it is certainly to be held as an excellent alternative.

As it is known that the method of killing a grasshopper influences the cellular pictures obtained with fixation, the birds were killed with ether and chloroform as well as by decapitation. No certain differences in effect between the two anaesthetics were observed, although the tissue from chloroformed birds seemed somewhat better. On the other hand, no marked distinction was noticed between the anaesthetized and the decapitated birds, although perhaps further observation may bear out the tentative opinion that in the tissues of the former the mitotic spindles are longer. If this is true, it will check with the results obtained with grasshoppers.

Forty-eight different methods of fixing bird tissues have been tried out in this study and 184 preparations have been made. The method outlined at the beginning of this paper has been repeated 157 times during the past seven years, so that we may be confident of the results obtained. Judgment of the fixation of sparrow testes produced with cold Flemming's solution had to be based on the general character of the results (preservation of delicate chromatic threads, lack of synizesis, etc.), as the gonads were quite mitotically inactive. There is some evidence in the sparrow preparations that spermatogenesis goes forward in waves, i.e., the testes would show principally one stage of maturation at a time. In my specimens the tubules are filled with spermatozoa which had apparently just completed maturation and no other stages of development were present.

The best of fixations will not make birds a classical subject for cytological investigations, as not only are the cells very small, but the chromosomes are numerous and the smallest ones almost beyond the limits of vision. Work on these forms is justified only to determine whether they fall into line with the cytological data obtained from so many other sources, with an additional incentive derived from the genetic evidence of sexual heterozygosity of avian females in contrast to the opposite condition existing in mammals and other groups.

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'DENTICLES' IN SHAPE AND ARRANGEMENT SUGGESTIVE OF SELACHIAN TEETH ON THE INNER SURFACE OF THE LIP OF THE CAT

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THREE PLATES

INTRODUCTION AND LITERATURE

The 'long, soft, villus-like outgrowths' which beset the lips of the human infant at birth are now well-recognized features. Ruysch figured them denuded of their epithelium in 1703. The thick investing layer is easily macerated and removed, revealing a striking display of slender 'papillae nervosae.' Without naming more specifically what appeared in his figure, Ruysch curiously introduced the term 'epithelis' for what he had taken away.[1]

In 1863 Luschka described the lips of the newborn as possessing two zones, the very smooth *pars glabra* extending from the red border as far as the line of contact of the closed lips, and beyond this line within the oral cavity, the *pars villosa,* bearing the villi.

[1] I am indebted for this historical note to Dr. F. T. Lewis, who points out that several German writers have been in error as to the original significance of *epithelium.* Thus Wetzel (Lehrbuch der Anatomie für Zahnärzte, 3 Aufl., Jena, 1922, p. 36) states that Ruysch's original reference was to the delicate skin of the nipple, as he thinks is implied in the Greek derivation. Hyrtl likewise in his Onomatologia anatomica (Wien, 1880, p. 205), citing Lélut, has it that Ruysch coined the term for the epidermis covering the papilla mammae . . . . ; 'zuerst an der Brustwarze,' inserts Virchow in the 4th ed. of his Cellular Pathologie ('71). But Ruysch, in describing Fig. 4, Tab. 4, of his Thesaurus Anatomicus I, both in 1701 and in subsequent editions, used epidermis, and not his term epithelis, for the covering of the nipple and its areola. The name epithelium, in fact, marks the discovery of the structures which we are endeavoring to interpret (Ruysch, Thes. Anat. III, 1703, No. XXIII, Tab. 4, Fig. 1; also Thes. VII, 1707, No. XLI, Tab. 2, Fig. 5).

Wertheimer ('83), following Klein ('68, and in Stricker's Handbuch, '71–'72), divided the lip of both newborn and adult into three distinct portions: the skin, which ends externally where the integument changes color; the transitional portion, which comprises all the red part of the lips visible when they are in contact, and the mucomembranous portion. The transitional corresponds to the pars glabra, the muco-membranous portion to the pars villosa, of Luschka and others. This third and innermost portion is thickest of the three—though translucent, it is much thicker than skin—and gradually passes, within the mouth, into the ordinary mucous membrane.

Blaschko ('87) stated that although the roughened villous appearance of the inner or hinder zone disappears during the first year, that zone may still be recognized in adult lips by features seen in sections.

Loewy ('91), a student of Blaschko, introduced a new method for the study of the epithelium covering the lips. He removed the macerated epithelium after staining it with hematoxylin, and examined its under side both dried and in glycerin. Upon the basis of the arrangement of certain folds thus revealed, he ascribed five zones to the lips, including the three previously recognized by Wertheimer. The other two are of transitional nature and are not important from the standpoint of the present study.

Remy ('89), without discussing the zonal arrangement of the lips, pictured the thickened epithelium and the tall papillae of the red portion.

Neustätter ('95), as did Blaschko earlier, found that in the child and even in the adult, the pars villosa, possessing thicker epithelium and larger dermal papillae, remains distinguishable from the pars glabra, though the papillae themselves disappear soon after birth.

Stieda ('00) described the development of the villi on the lips of the human embryo.[2]

[2] Schumacher ('24) figures the villi in a human embryo of six months (cf. p. 97.

Ramm ('05) showed that the villous zone is not restricted to the lips, but extends backward as a band from the corner of the mouth along the inner surface of the cheek. She did not discuss the original extent of the embryonic mouth, which we understand to be relatively a much wider cleft than in later stages, so that the buccal band of villi may be regarded as primarily oral. A critical study of this relationship is still needed.

Watt, in 1911, found that the zones described by Neustätter as persisting on the lips throughout life likewise remain distinguishable in the villous band of the cheek. In other words, there is in this band a pars villosa, remaining readily distinguishable from the zones above and below it by the possession of thicker epithelium and of larger, more vascular, papillae. Watt thought that the villosa represented the entire extent of the original mucosa of the lips and cheeks, the smooth portion being added by spreading from the gums, but he offered no evidence in favor of this interesting conjecture. He made fragmentary observations on "an adult collie dog and a fetal Guinea pig," which showed in both cases the existence of the specialized buccal band extending from the corners of the mouth. No villi were associated with it in the Guinea pig and apparently there were none in the dog, where, however, the buccal band itself was more distinct than in man.

At this point the older literature in comparative anatomy becomes relevant, for the question arises whether these processes are, strictly speaking, oral, or merely local representatives of features found on the tongue and down the oesophagus, being perhaps even analogous to intestinal villi. Owen ('68) reviewed the occurrence of papillae in the lower vertebrates as follows:

> In certain sharks "numerous pyramidal retroverted processes, jagged and fringed at their extremity, project from the inner surface of the oesophagus." In the basking shark near the cardia they "acquire unusual length, dividing and subdividing as they extend inwards, so that the cardiac opening is surrounded by ramified tufts

directed toward the stomach.'' In turtles (herbivorous species only) the lining of the oesophagus bears ''pointed processes, directed to the stomach, and covered by thick epithelium.''[3] In birds, the laryngeal aperture is unprovided with an epiglottis, ''but is defended by retroverted papillae at the base of the tongue.'' The upper surface of the mouth is also ''commonly provided with retroverted papillae, and similar mechanical helps to the right course of the food occur at or near the fauces.''[4] ''In birds the mucous coat of the oesophagus is rarely villous.'' It is so in the ostrich, however.

On the buccal mucous membrane of ruminants the villi are hard and retroverted, and of extensive distribution; ''those on the inside of the lower lip are large and pointed, interspersed with groups of mucous follicles.'' ''Along the roof of the mouth they are flattened, and disposed in parallel transverse rows with retroverted denticulate margins; the papillae usually attain great size inside the cheeks, opposite the masticating line of the molars.'' ''In the camel they are aggregated and obtuse''; in the giraffe ''strong, retroverted, pointed, some of them half an inch in length.'' In the act of rumination, the regurgitating bolus is driven into the mouth with force; the papillae offer mechanical obstacles to its escape, and also serve to confine the finely comminuted vegetable substances to the molar region during the process of mastication, according to Owen. In the rodent, Capromys sp., there is a single fleshy papilla situated on the inner surface of the upper lip; three hard white tubercles are present on the palate.

[3] I have examined the extraordinary horny cones, some of them 6.5 cm. long, which occur mixed with small ones throughout the lining of the oesophagus of the leatherback turtle. This specimen, 6 feet long and weighing 474 pounds, was supplied by the U. S. Bureau of Fisheries. After preservation in alcohol the softer tissue in the interior of these cones macerates and the cornified outer layer comes away retaining the shape of the original 'tooth'. Though soft at the base, this epithelium is dense at the tips where it forms a stiff sharp point. The epithelium of the villus, as observed in sections, consists of an outer corneous layer, in which cell outlines are lost, and an inner layer, eight or nine cells in thickness. In the core of the villus a stratum of connective tissue lies just beneath the epithelium; it is made up of fibers densely packed, their long axes running lengthwise of the villus. These fibers swing across at the base of the villus, enclosing the pulpy central portion, in which fibers pass principally crosswise, and form a meshwork of much looser texture.

[4] In a series of studies upon the head region of amniotes edited by Fleischmann, Aulmann ('09) pictures the palatal papillae in the common fowl; papilliform structures are not described or figured by Thäter for the snake or the turtle.

The conspicuous buccal villi of the domesticated ruminants are described as to their gross appearance and distribution in most of the veterinary anatomies—Chauveau ('05), Sisson ('10), Martin ('12–'19), and Ellenberger and Baum ('15). Schulze ('12) published a special paper about them, and goes further than Owen in discussing their function. He finds that the pasty mass of food returned from the paunch to the mouth is pushed forward by the tongue into the space between the teeth and the lips; there it is guided laterally by the backward-directed papillae of the lips and cheeks to the sulcus near the end of this space. Then, by the action of the muscles of mastication and of the cheek, the bolus is pushed medially between the teeth to be crushed and pulverized. In ruminants, therefore, the buccal villi are highly developed and seem to have acquired a special function.

In the pig Ellenberger and Baum mention a large patch of high, soft, matted papillae in the tonsillar area.[5]

The villi in rodents have been studied by Schulze ('16) and recently by Schumacher ('24), who finds a group of six spinous papillae within the cheek of the mouse, and nodular outgrowths on the buccal mucous membrane in the Guinea pig. Such structures apparently do not occur in the rat or hare.

Villi in the carnivora have received little attention. In the dog Sisson describes the long, soft villi occurring "on the lateral walls of the isthmus faucium." The cat is included in the list of animals studied by Chauveau and Ellenberger and Baum, but no mention is made of the existence of villi on the lips. Reighard and Jennings, however, in describing the upper lip of the cat, record that "for some distance on each side of the frenulum the inner surface of the lip bears numerous papillae." Other manuals of dissection have been examined, but no similar reference has been found. In fact, although the literature has been quite carefully

[5] The writer has recently found, in forty specimens of adult hogs examined at an abattoir, that a low and rather tag-like projection is invariably present on the buccal mucous membrane opposite the grinding surface of the molar teeth. One or two even shorter members may also occur.

searched, this remains the single reference encountered that deals with the existence of such structures in the cat.[6]

Immisch ('08) studied the lingual papillae in various domesticated mammals, and was led to regard the labial and buccal villi, when such occur, as merely extensions of the lingual structures. Evidently he failed to find them in the cat, for he writes, "All the domesticated mammals have on the tongue, in addition to the gustatory papillae, or *papillae gustatoriae,* the mechanically operative papillae, *papillae operariae;* only the ruminants possess such also on the buccal surface of the lips, the cheeks, and the sub- and paralingual areas of the floor of the mouth."

From the literature thus far cited it appears that the papillae operariae can hardly be set apart from others not mechanically operative. Such structures are much more widely distributed than Immisch supposed. Schulze found them in marsupials, namely in four species of Macropus. In them he described, with an elaborate Greek nomenclature, various subdivisions of the lip which may be comparable with those in man, though he makes no reference to such a possibility.[7] Along the free border of mucosal ridges within the lips and cheeks "there may be spinous or conical processes which generally stand in a row and have pointed ends." But since he finds that "the occurrence and the formation of these spines is not always alike in the different species," he concluded that they are therefore "as a rule to be regarded as only a specific character." If, however, labial villi can be identified in many types of mammals from marsupials to man, they are presumably a morphological feature of much wider significance.

[6] On inquiring of Professor Jennings the source of the statement, he very kindly replied that he had read my note in Science upon this subject with interest, and had forgotten that his book referred to these structures, so that he was of the opinion that the sentence in question came originally from Professor Reighard. Professor Reighard wrote that he did not recall any reference to the papillae in the literature, and that he doubtless noted the papillae during dissections, as an original observation. Neither he nor Professor Jennings studied or figured these structures, but they have recorded their existence in the sentence which I quoted in full.

[7] Especially see Schulze's figure 2.

In the following papers comparisons with denticles in fishes are introduced.

In a recent paper, West ('25), discussing the significance of the human segmented gums which at birth are sometimes mistaken for erupted teeth, includes also a description and excellent plates of the villi of buccal mucous membrane, gums, lips, and palate. He finds that the villi appear first, and are subsequently most luxurious in their growth, on the "dorsal extremity of the buccal mucous membrane." They are, however, most obvious on the lips and gums, in which locations they occur synchronously. On the lips the villi undergo pronounced increase in length, with the result that they become long and almost filamentous. On the gums a series of changes occurs which is closely comparable to that described for the development of the denticles in the cat (Anson, '24). The surface of the gum is at first merely roughened; it next becomes nodular; some of the nodules then elongate and become 'free villi,' their shape resembling, to a striking degree, that of the long pointed denticles. So close, in fact, is the resemblance of the two, that one could with difficulty distinguish the 'free villi' (West's pl. III, fig. 29) from the spine-like denticles present typically in old cats.

"In considering the villi of the lips and gums," West remarks, "one is at once struck by the resemblance which they bear to the condition found in the mouth of Petromyzon. In this creature the villi are said to contain sensory nerves, to be suctorial in action, and to assist their possessor in anchoring itself to the belly of the fish upon which it preys." These villi of Petromyzon, to which West refers, should not be confounded with its horny teeth. In regard to the possible function of the villi in the human embryo, West mentions that Corning ('21) thought that they produced an air-tight contact in suckling, and that Neustätter ('95) and Ramm ('05) believed that the double zone, even after the disappearance of the papillae, served a similar end.

Shortly before the appearance of West's paper Dr. Frederic T. Lewis, at the Buffalo meeting of the American

Association of Anatomists, had suggested a very different interpretation; he displayed photographs of the labial 'villi' in man, and others of the teeth of the skate, both entire and sectioned, and proposed to regard them as homologous structures. That is to say, he considered the human labial villi, "notwithstanding their small size, their tendency to point outward and their lack of dentine, as actual denticles or potential selachian teeth."

In an earlier paper the writer reported briefly the occurrence of 'bilaterally arranged papilliform structures' on the inner surface of the upper lip of the cat. They arise as do those of the human embryo from an inner zone, which lies above the free margin of the lip, in front of the dental ridge and gums. These, it was then stated, are the "equivalents of the villi of the human lip, though the inner zone which they form is not exposed at the oral margin," but lies wholly within the lips. Can the papilliform structures of both cat and man be regarded as homologous with the denticles in the shark? Such an interpretation invites, of course, study of the possible relation of these outgrowths to the true teeth of higher vertebrates, the fate of this productive zone in the several classes of vertebrates, and the phylogeny of the lip. These problems will be the subjects of further study. The present paper is limited to the development of the labial villi in the cat, the morphological changes which they undergo with age, and the question of their relationship to certain structures in the oral region of the lower vertebrates.

I conducted this investigation in the Embryological Laboratory of the Harvard Medical School, while holding a Medical Fellowship from the National Research Council. To the donors of this Fellowship I wish to express my grateful appreciation, and also to Doctor Lewis who placed his notes at my disposal.

MATERIAL AND METHODS

Twenty-six specimens of embryos of the cat cut in serial sections were available in the Harvard Embryological Collec-

tion. They ranged in length from 4.6 to 39.0 mm. To supplement these younger stages ten older specimens were selected from a large stock preserved in alcohol. Those chosen were five sizes, measuring, respectively, 42.5 mm., 59.0 mm., 74.0 mm., and 87.0 mm. Two individuals of each of these sizes were taken; the lips of one were cut in serial sections and those of the other retained for binocular examination. For the postnatal stages 138 kittens and cats were examined, including specimens from six days of age (150.0 mm.) to very old individuals (520.0 mm.). From this collection thirty-two specimens received more careful study, in a manner to be described later. Although the villi are evident in the fresh specimens, it was found advisable to remove the lips and place them immediately in Zenker's fluid or Bouin's fixative; they were run up through a graded series of alcohols to 80 per cent, and were examined with a binocular dissecting microscope. Certain specimens were selected for sectioning; the sections were cut 10 μ in thickness and stained with hematoxylin and eosin.

The descriptions will concern only the upper lip, since in the cat the papillae are limited thereto, being absent so far as was observed, within the cheeks, on the lower lip, and over the gums. Unless otherwise stated, the structures on one half of the upper lip alone will be discussed.

Certain terms denoting directions and relationships will be used regularly throughout the text. *Dorsalward* will denote direction toward the dental side of the papilla-covered zone; *Ventral* toward the labial side, i.e., in the direction of the free margin of the lip. *Lateral* will mean toward the corners of the mouth; *Medial* toward the midline of the lip; *Horizontal* will mean in the plane of the oral cleft; *Vertical* will be sagittal or parasagittal, at right angles with the horizontal.

In this account, papilliform outgrowths in the lip of the cat will be referred to as *'villi'* or, provisionally, as *'denticles.'* The connective tissue sometimes invading the villus will be called a papilla. The name *villus,* introduced

in Luschka's fundamental paper, and established through his term *pars villosa labii* is not very appropriate for low, stunted growths, sometimes plate-like, and seldom if ever hair-like, but the name seems to be already established.

OBSERVATIONS

A. Young embryos: 4.6 mm. to 39.0 mm.

Microscopic examination of sections. The specimens, ranging in length from 4.6 mm. to 39.0 mm., were examined in search of the early stages in the development of the villi. No evidence of their presence was noted.

B. Older embryos: 42.5 mm. to 87.0 mm.

Binocular examination of whole lips. The villi make their appearance between the 39.0-mm. and the 42.5-mm. stages. In the 42.5-mm. embryo, on the inner surface of the upper lip, a low ridge extends horizontally and laterally from a point near the midline, to end somewhat less than halfway to the corner of the mouth. The ridge is not simple, but presents three subdivisions in the form of low nodular outgrowths, arranged horizontally in linear series, these low hummocks representing complete and almost equal subdivisions of the ridge.

In the embryo of 59.0 mm. the ridge as a whole has become considerably enlarged. Extension, however, occurs only in the ventral and lateral directions, the dorsal limit of the ridge remaining, apparently, relatively fixed. The dorsal, which is also the original, part of the ridge undergoes, with extension, a synchronous process of subdivision whereby three new villi are added to the lateral end of the row. The dorsal row now consists of six members whose apices have begun to point ventralward. Nine additional villi, low and nodular, dot the newly formed ventral area of the ridge; they exhibit no regularity of arrangement, and, because the process of outgrowth has not kept pace with that of extension, they are separated from one another by appreciable spaces.

They are all formed later than those in the dorsal row, and are located on the labial, and not on the dental side of the latter group. Thus, at this stage, there are in all fifteen villi on a lateral half of the upper lip. No more than three of these are arranged in any vertical or paramedian plane.

In the embryo of 62.0 mm. the number of villi has increased to seventeen. Another member has been added to the dorsal row, which now consists, therefore, of seven denticles. They have become broader, somewhat longer, and have begun to point slightly downward. The new lateral member of the group is continuous with an undivided portion of the ridge. No striking change, affecting either arrangement or shape, has occurred among the nodular denticles which cover the labial portion of the ridge.

In the 75.0-mm. embryo the dorsal portion of the ridge is completely subdivided and bears eight villi. Toward the free margin of the lip nineteen nodular villi are scattered over the ridge with no certain arrangement. Some of these seem to be formed by division of a larger individual, by a process perhaps analogous to that observed in the origin of villi from the dorsal ridge. A few still present the appearance of low hummocks, indicating that the formative period extends through this stage. As many as four denticles may now be included in a vertical row.

The 87.0-mm. stage is characterized not by further increase in the number of villi, but rather by a very striking change in the appearance of the papilla-covered area. The change is produced by the growth and turning downward of many of the denticles. At this stage their continued backward growth seems to be prevented by the presence of the gums, and they turn downward, pointing toward the free edge of the lip, and describing as they do so a right-angled bend. Elongation is not limited to any group or tier, although it does affect earliest and most regularly the members of the dorsal row. Lengthening is accompanied by widening, with the result that the denticles become flattened, bluntly pointed, and incisor-like. Shapes intermediate between this and the

earlier or nodular type are not uncommon. The latter are often obscured by the longer members which overarch them from above, imparting the superficial effect of a diminution in numbers. Actual decrease in numbers is, however, characteristic only of the advanced adult stages.

The process of elongation, here observed in its beginnings, gives forewarning of the condition that obtains in the adult, namely, one in which denticles, long and pointed, or even resembling spurs or spines, overlap, and, in surface view at least, partially or wholly conceal those of the lower tiers. They never become as thin, attenuated, or thread-like as do the villi of the human lips. It even seems probable that some denticles remain low and dome-shaped, since similar structures are found in the old adults. However, the hummock-like character of these may be secondary, i.e., a step in a retrograde process of flattening and eventual disappearance.

On account of the unequal growth of the villi, disposal in tiers is not clearly shown in specimens of the 87.0-mm. stage. In the newborn it is more evident. The members of the tiers or horizontal rows usually stand directly in front of or behind one another, forming vertical rows which may be close-set. In other words, the denticles in successive horizontal rows are not imbricated.

Microscopic examination of sections in embryos. In sections of the lip of the 42.5-mm. embryo the epithelium covering the developing villi is thickened, being six to seven cells in depth, whereas the surrounding epithelium is not more than three cells in thickness. Beneath the basal layer of epithelium there is a concentration of mesenchymal cells. Subsequent outgrowth brings about an inclusion of the mesenchymal cells so that they form a core for the developing villus. Within this core ramify branches of small blood vessels. In serial sections of lips from embryos of 58.5 mm., 60.0 mm., 75.0 mm., and 85.0 mm. the concentration of mesenchymal cells is even more striking. Activity of the stratum germinativum and proliferation of mesenchyma are

doubtless linked with the abundant vascular supply of the area.

The subdivision of the ridge into nodular outgrowths seems to be associated with the extension of arterial branches outward to certain points located peripherally. The cellular activity accompanying this increase in vascularity is expressed in the outgrowth of villi. According to this view, the villi would be formed, as in the development of the denticles of elasmobranchs, by a marked proliferation of the cells of the corium, this aggregation of cells carrying with it the basal layer of epidermis. The sections do not reveal a conversion of the mesenchymal cells into osteoblasts or of the overlying ectodermal cells into an enamel organ. It is not clear from a study of the sections now available that differentiation in either of these directions is even initiated.

C. The villi after birth

Binocular examination of whole lips. In the newborn the villi are about thirty in number on either lateral half of the upper lip. They are similar in shape to those described in the 87.0-mm. embryo. More of them have become elongate and, as some partially protrude from beneath those next above, they form rather poorly defined rows. They are approximately 0.5 mm. long and 0.25 mm. wide at the base. They usually point ventrally or outward and slightly toward the midline. In this, as in all other stages, a portion of the upper lip in the direction of the corners of the mouth is quite smooth and devoid of villi. In addition to these villi, all of which have taken origin from a horizontal ridge in the manner described, there is in the newborn a median group consisting of a single row of four rather large incisor-like papillae. Of these four, the two next the midline are largest, measuring 1.0 mm. in height and 0.75 mm. in width at the base (cf. fig. 1). In later stages they alone—of the four—remain prominent, as large mounds on either side of the cleft which separates the halves of the lip. The lateral two become so reduced in the process of growth and expansion of the lip,

that in later stages they are no longer noticeable. A comparison of figures 1 and 3 will serve to illustrate these changes.

The villi of the lateral groups in the stages after birth may be described as of four types: 1) short, bluntly conical villi, whose height is approximately equal to their width at the base; 2) short, flattened villi having much the shape of human incisor teeth, though relatively thicker; 3) long cylindrical villi with rounded free end; 4) long, conical, sharply pointed villi, which may become almost spine-like. In addition to these four types of definitely formed villi, there are certain questionable structures, notably a ledge-like projection located just below the most ventral villi, which acquires prominence in kittens of about 250 mm. This is well shown in sections (fig. 5) from older stages. It may project into the oral cavity as far as the largest villi. It is not a simple ridge or ledge, but its summit exhibits subdivisions or perhaps irregularities, some of which resemble low, broad villi. These mounds or blocks pass by gradations into villi. It is conceivable that this zone of the lip, intermediate in position between the exposed margin that produces hair, and that within the mouth which gives origin to true villi, is of transitional character, and hence incapable of producing denticles of the regular type. The ledge and blocks may be reminiscent of the earlier existence of additional rows of denticles.

Such blocks occur, likewise, in the villous area. They may represent areas of papilliform structures whose evolution is incomplete, or perhaps villi which have been flattened through the failure of the process of growth to keep pace with the expansion of the lip. The blocks are either low, broad, and button-like or bluntly truncate. Toward the lateral portion of the groups, long pointed papillae grade into those of more blunt shape, and these, in turn, into the flattened blocks (fig. 3).

In the young kittens the small nodular villi already described for the embryos are often present, indicating that the formative period extends even beyond the stage of the newborn. However, the short, flattened villi predominate.

This type is illustrated in figure 1. These blunt, more or less compressed villi, common to the younger specimens, begin early to decrease in number.

In the specimens of medium size the number of villi has decreased, but the loss is accompanied by an increase in size and, as a rule, by the acquisition of a more definitely pointed character in those which remain. As in the early stages, they usually point outward; however, instances are not rare in which they stand erect and project directly backward into the oral cavity. In the process of elongation the villi may either retain the original blunt shape and merely become longer, appearing finally as cylindrical outgrowths with rounded end, or, on the other hand, and this is much more commonly the case, they may become not only elongate, but sharply pointed (fig. 3). The largest attain a length of 2.75 mm. and a breadth of 1.25 mm. at the base. Flattening occasionally accompanies the other changes.

In the oldest specimens the long pointed denticles alone are common (fig. 3). The cylindrical papillae seem to be transitory; possibly through a similar process of elongation and change of shape, though tardy, the cylindrical villi regularly add themselves to the ranks of the long pointed type. But, if so, certain of the pointed forms must at the same time drop out, for the increase of this type is relative and not absolute. All forms apparently become involved in the general reduction which occurs in old age.

This process may progress so far that, in old adults, barren areas supplant those which formerly bore villi. A great deal of irregularity and asymmetry between the halves of the lips, affecting size, shape, number, and arrangement of villi, characterize this final stage. The long pointed villi which remain may form a cluster of from four to eight constituents, whose apices are directed toward a common point lying beyond the limit of the lip. They apparently represent the persisting members of the original dorsal row, now projecting over an area from which the denticles have largely disappeared. Another striking effect is produced when the

long villi are so arranged as to form a single dorsoventral series, the only others present being of block-like shape. The individual villi of large size may bear upon the basal portion as many as four small scale-like eminences which resemble cusps or accessory denticles; similar small villi may occur in the neighboring areas. Large villi may be fused at their bases, appearing as one with bifid or bilobed extremity. The surfaces are always smooth, presenting no corrugations or grooves.

Sometimes the arrangement of selachian teeth is strikingly reproduced, at other times there is total lack of order. The typical relation between size of specimen and number and shape of villi may be occasionally departed from, so that, in old specimens, types that are usually associated with much younger specimens sometimes occur, and vice versa.

Microscopic examination of sections in specimens after birth. At birth the epithelium covering the villous area is three to five times as thick as that over the exposed portion of the lip. This is shown in figure 4. The deepest cells, composing the stratum germinativum, usually form a single very definite layer; they are elongate, with their long axes arranged perpendicularly to the surface; they stain deeply, and occasionally exhibit mitosis. This layer seems to rest directly upon the connective tissue below, without the intervention of a specialized basement membrane. There is still no indication, so far as observed, of the presence of enamel or allied substance, at the junction of epithelium and connective tissue, or of differentiation of the basal cells into an enamel organ. Several strata of cells, of rounded or polygonal outline, succeed the basal layer, and stain less deeply than the basal cells. As in stratified epithelium generally, these cells have intercellular clefts or channels between them, bridged by the fibrils which pass from cell to cell. In the succeeding superficial layers, the cells become progressively more flattened. They have well-defined, but irregular, wavy boundaries; the intercellular spaces are no longer evident. Although eleidin granules are absent, the

cells appear to be more or less cornified. The cytoplasm is homogeneous and of waxy appearance; nuclei are rare in the outermost layers, and those that do persist are usually pycnotic and shrunken. The cores of the villi are made up of bundles of connective tissue, among which capillaries ramify. There is no epitheloid layer or other evidence of differentiation into bone-forming cells.

In older stages the layering of the epithelium continues to be less complex than in the adjacent epidermis, resembling, in fact, mucous membrane rather than skin. In the cells of the intermediate layer of the epithelium the intercellular structures, namely, the clefts and the bridges, become more pronounced. The connective tissue forming the corium is somewhat more densely packed. Vascularity remains abundant (fig. 8), even into the advanced stages and is perhaps associated with their continued growth. One striking feature—besides an increase in size—readily distinguishes the villi of the adult animal. This is the appearance, at about the 290-mm. stage, of true dermal papillae in the basal layer of the epithelium forming the denticles. They spread inward from the haired zone of the lip, and, by gradual extension, finally involve even the denticles farthest removed from the margin of the lip (fig. 5). This seems to represent the encroachment of features of the adult epithelium upon the area of the denticles. The inroad of dermal papillae results, as if through compression, in thinning of the epithelial layer covering their tips. It is worthy of note that the villi cannot be confused with the dermal papillae, the latter appearing very much later. The dermal papillae, of which as many as thirty-three have been observed in a single section of a large villus, are obviously secondary structures.

SUMMARY AND CONCLUSIONS

In the cat, so far as observed, the villi are restricted in their distribution to the inner surface of the upper lip. They appear in the 42.5-mm. embryo as bilateral groups, each composed of three members, arranged in a horizontal row.

With growth of the lip, the ridge which bears these villi extends laterally, adding new villi to the original row, and also ventrally, where others arise distributed irregularly. At first, corresponding to the direction of growth, the villi are pointed directly backward into the mouth cavity, their long axes being at right angles to the inner surface of the lip. As if encountering an obstruction in the presence of the developing gums, they turn downward, and so point out of the mouth, which is the reverse of the direction taken by selachian teeth. The villi are most numerous at about the time of birth, numbering twenty-seven to thirty on each side, or between fifty and sixty altogether. They are arranged in four ill-defined rows. In later stages the villi tend to disappear, some adult specimens possessing as few as five on one half of the lip. The villi that remain, however, usually enlarge and lengthen, becoming finally slender cones.

Microscopic sections reveal an epithelium much thickened over the villous zone and composed of strata characteristic of mucous membrane. There is no evidence of the formation of an enamel organ. The core of the villus in the embryo is composed of mesenchymal cells, in which capillaries ramify. The mesenchyma exhibits concentration, but gives no indication whatever of bone formation. Subsequently, this mesenchyma becomes a loose connective tissue which remains vascular even into the adult stages. The connective-tissue cores of the villi, covered by the layer of thickened epithelium, are not to be confused with true dermal papillae, which appear much later, are relatively small in size, and gradually invade the basal layer of the epithelium covering the villus. Back of the gums of the cat, which are themselves destitute of villi so far as was observed, there are two sorts of structures more or less villous—the lingual papillae, which Immisch regarded as spreading to the cheeks (and lips), and the palatal ridges and spines, which are very strikingly developed in the newborn kitten. There are seven of these palatal ridges, curved in outline with their concavity directed posteriorly. Just within the incisor area of the gums, a number of nodular

papillae are distributed without definite pattern. Posterior to this group, a rather regular series of ridges and spines, or serrations, is encountered. All of these are pointed inward and backward. They present the following typical arrangement: a row of spines springs from the base of the first ridge and partially overlaps it; then comes the ridge followed by two rows of spines, the anterior one of which is overlapped by that ridge, and the posterior partially covering, in turn, the succeeding ridge. The rows following the sixth and seventh ridges are poorly defined. The fifth, sixth, and seventh ridges possess serrated crests. The ridges increase in lateral extent from before backward, the increase corresponding to the greater width of palate and of buccal cavity. The largest of the spines are 0.55 mm. in length and 0.25 mm. in width at the base. They are flattened in the same plane as the ridges. The rows of spines consist of from fourteen to twenty-six members.

Microscopically, the spines and ridges resemble villi. In the early stages of their development they show vascular cores of connective tissue extending into the stratum germinativum of the epithelium. Even before a mounding of the epithelium has been produced, the direction of extension of the core is very definitely backward. In this regard they differ from the papillae of the lip, in which the deviation in direction of growth from a right angle to the surface occurs only after the papillae have attained appreciable length as free projections. Neither the lingual papillae nor the palatal ridges, nor the effective oesophageal teeth of the turtle seem to possess the dental characters of the labial villi. To be interpreted as denticles the questionable structure must be an elevation formed of mesoderm and ectoderm, tooth-like in form and situated in dentigerous territory—that is, between the true teeth and the external skin. Defined in this way, the human labial villi, those of the monkey[8] and of the cat,

[8] The monkey is included on the basis of a single specimen kindly sent me by Prof. G. S. Huntington, of Columbia University. This was a Semnopithecus entellus, preserved "probably at term or shortly after." It was found that the

and apparently the buccal villi of ruminants if they develop along the original extent of the embryonic mouth, may be considered as possible denticles. These structures, though entirely destitute of bone in so far as hitherto observed, are nevertheless not comparable with the horny teeth of cyclostomes. As described by Warren ('02), such horny teeth come from cells within the epithelial layer and just above the mesodermal papilla, which proliferate, become granular, and finally corneous and homogeneous. As a cone, they compress, and then rupture the overlying epithelium, breaking through as the horny tooth. Thus, the tooth is made up of cornified cells, developed within the epithelium, and carries no contribution from the mesoderm. In this they differ radically from the labial villi.

In the selachians the horny tooth is replaced by one of a calcareous nature (Owen, '40; Hertwig, '74; Dean, '23). The denticle begins, not as a modification of cells within the epithelial layer, but as a cone of dentine deposited by cells of the mesoderm. The surface layer of the denticle, always lying just below the basement membrane, becomes covered by a cap of hard enamel-like material deposited by the overlying ectodermal cells. The tooth owes its origin primarily to the concentration and differentiation of cells of the mesodermal core, the contribution of the ectoderm being made subsequently. The core itself is, in the case of the denticle, of prime importance. In pointing out a possible connection between the teeth of cyclostomes and selachians, Warren ('02) mentions that there exists among fishes, living and fossil, dentines exhibiting every transition from irregular

upper lip bears numerous, short conical villi. On the lower lip the short type predominates, but toward the midline longer villi are common—none, however, as attenuated as those found on the lips of the human newborn. On the lower lip a smooth area intervenes between the median villous area and the narrow band of villi that extends laterally along the cheek from a point just ventral to the corners of the mouth. Histologically, the villous zone of the monkey is closely similar to the same zone in man. The epithelial thickening of the pars villosa is very pronounced—the epithelium in this area being approximately fifteen times as great as that of the epidermis over the exposed margin of the lip.

masses of vascular dentine to highly organized types, and enamels showing every transition from thin 'varnish-like' layers of 'apparently homogeneous calcareous matter' to thick layers of 'striated prisms and interprismatic substance.' Both dentine and enamel may, therefore, vary considerably in amount.

With the denticles of selachians as thus described, the labial villi have a definite structural resemblance which is further illustrated in plate 3. Figure 6 is the section of a dermal denticle of a Spinax embryo as published by Lasser ('03). Similar in form and constituents, but lacking the seam of dentine, is the villus of the cat, photographed in figure 8; and Stieda's somewhat diagrammatic drawing of the human labial villus here reproduced as figure 7 is equally comparable.[9] The total absence of enamel or dentine from the labial villi is a feature not to be slighted and makes our interpretation somewhat venturesome. Yet embryonic teeth are altogether lacking in bony elements, and it is not unreasonable that in these abortive structures growth in size has occurred without a corresponding differentiation of tissue.

A more serious objection, perhaps, lies in the position of the labial villi in relation to the jaw. They are not placed along the inner surface like the developing teeth in the dogfish embryo, but are out in front of it, correlated with the development of the lips which are considered altogether lacking in the fish. Yet the relation to the cartilage or bone of the jaw is not a criterion by which teeth can be recognized. In some species of Selachoidei, as Imms ('05) has pointed out, the denticles may be distributed uniformly over the mucous membrane of the mouth, pharynx, and branchial arches, as far posteriorly as the beginning of the oesophagus; in others, they have been lost altogether over these areas;

[9]Although elongation may be relatively greater in the case of the human villi, groups of conical villi do occur (West, '25, pl. III, fig. 29, lingual surface of gum in embryo of forty weeks), which resemble to a remarkable degree the type which predominates in the oldest cats. The large villi of ruminants with their basal layer indented by dermal papillae are scarcely distinguishable in sections from those of the cat.

many intermediate stages in distribution are represented by other species. With the evolution of special jaws he believes that "the denticles would tend to become restricted to them, and constitute ordinary teeth, the residue of the stomodeal invasion of dermal denticles would become pharyngeal teeth, or gillrakers, or remain as vestigial structures or vanish altogether."

In mammals the figures which we have presented seem to show that the denticles have not vanished altogether. In the oropharynx of mammals the ichthyoid plan of development is preserved, therefore, not merely in the persistence of gill clefts. The pentagonal shape of the mammalian mouth, as pointed out, for example, by His and Lewis, is fundamentally that of fishes, and within it in the cat, as shown in our figures better than in any description, there is an extraordinary array of tooth-like formations. Believing that such structures might be present in the cat, and unaware that anything of the sort had ever been observed, we found them in the location expected. That is the first step in establishing our interpretation, but a further one is needed, namely, to determine the intermediate conditions and homologies which should be found in the amphibia. A study for this purpose is now in progress.

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PLATE 1

EXPLANATION OF FIGURES

Photographs of the inner surface of the upper lip; three specimens, all magnified 3⅓ diameters. In every case the free margin of the lip is directed downward.

1 Kitten, 290.0 mm. Inner surface of upper lip, showing blunt, flattened villi.

2 Kitten, 295.0 mm. Inner surface of upper lip, showing pointed villi.

3 Cat, 440.0 mm. Inner surface of upper lip. Some villi are cleft, others surmounted by small scale-like members. The ledge ventral to the villi is prominent and partially subdivided into blocks. Small irregularities appear on the exposed margin of the lip.

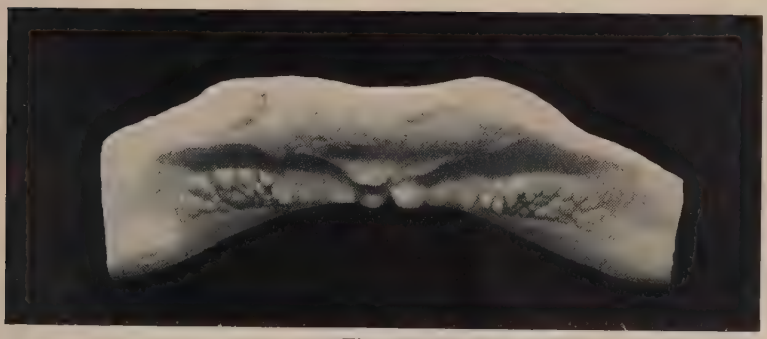

Figure 1

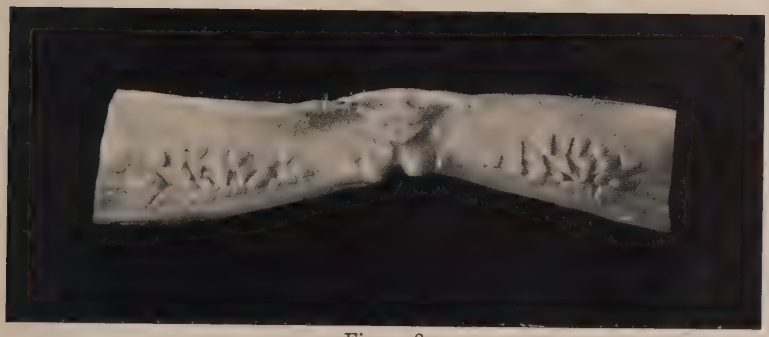

Figure 2

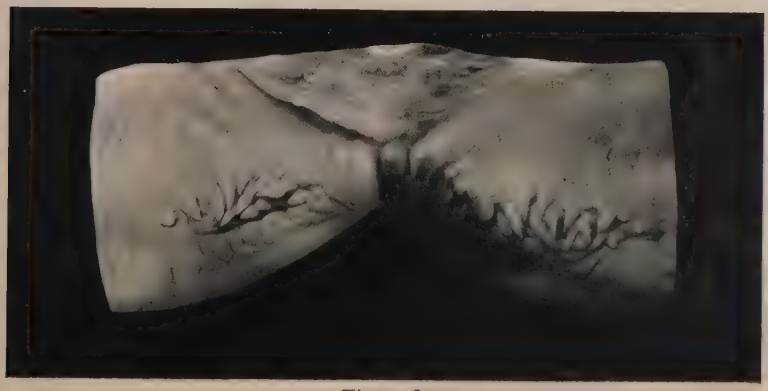

Figure 3

PLATE 2

EXPLANATION OF FIGURES

Photomicrographs. Sections of upper lip. Same magnification—25 diam.

4 Newborn, 117.0 mm. Section through the upper lip, showing blunt villi, with their apices directed downward.

5 Cat, 440.0 mm. Section through the villous zone, showing three large pointed villi in series, and, ventrally, the horizontal ledge or ridge.

Figure 4

Figure 5

PLATE 3

EXPLANATION OF FIGURES

6 "Hautzahn eines Spinax Embryo von 10 cm. Länge" (from Laaser, '03, Taf. XXVIII, fig. 8). Magnification, 200 diameters.

7 "Längsschnitt einer Zotte der Pars villosa." Newborn human (from Stieda, '00, Taf. III, fig. 3). Magnification, 150 diameters.

8 Photomicrograph. Kitten, 205 mm. Section shows long pointed villus and shorter villus; in the latter capillares are very evident. Magnification, 80 diameters.

Figure 6

Figure 7

Figure 8

ON THE FORM OF THE ANTERIOR HORN CELLS

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FOUR FIGURES

The large motor cells in the anterior horns of the human spinal cord are usually seen cut transversely. They are so illustrated in text-books of anatomy and neurology. The cells would seem from these illustrations and their accompanying descriptions to show no marked and constant difference between any of their three diameters. We have found that this is not the fact.

The present photographs (figs. 1 and 2) show that when the spinal cord is cut along its cephalocaudad axis, i.e., longitudinally, the cells have a very different form from that which is seen when they are cut transversely. In the cells which we have photographed the transverse diameters as measured are from one-fifth to one-fifteenth the length of the long or cephalocaudad diameter. The long diameters, i.e., cephalocaudad average about 100 μ. It is sometimes difficult to separate the actual cell body from its dendritic processes, but the location of the cell pigment assists materially in settling this question.

Wax reconstructions of cells (figs. 3 and 4) determine these facts with greater accuracy. When reconstructing cells from serial transverse sections, they seem to be shorter than when seen in longitudinal sections, because the extreme ends of the cell cannot be recognized accurately. As a rule, we have only been able to trace any given cell through six transverse sections (15 μ thick). In longitudinal sections cut in the

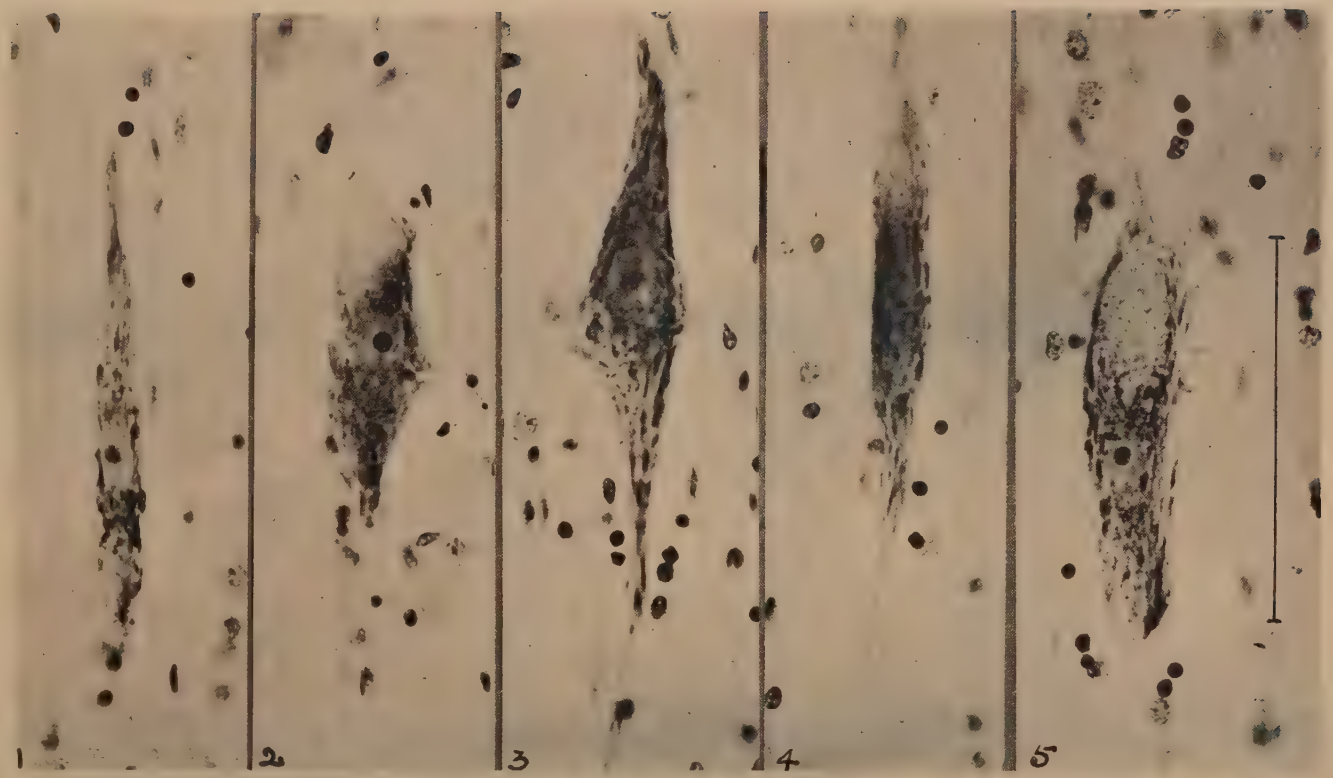

Fig. 1 Five anterior horn cells from the human thoracic spinal cords. × 550. Nissl's method with toluidin blue. Horizontal section. Scale equals 100 μ. Note, *a*) the great length in relation to the width. *b*) The variation in width and length depending upon whether the cell has been cut where a dendritic process is leaving (2 and 3) or to one side of the greatest transverse diameter (1 and 4).

Fig. 2 From the same section as figure 1. × 130. Scale equals 100 μ. To show the great length in relation to the width. The cell lying nearest the scale on the left has a mass of light green pigment at its right end and two dendrites at its left end. The relation of its long to its transverse diameter is 1 to 7. This gives an idea of the same relations in the other cells shown.

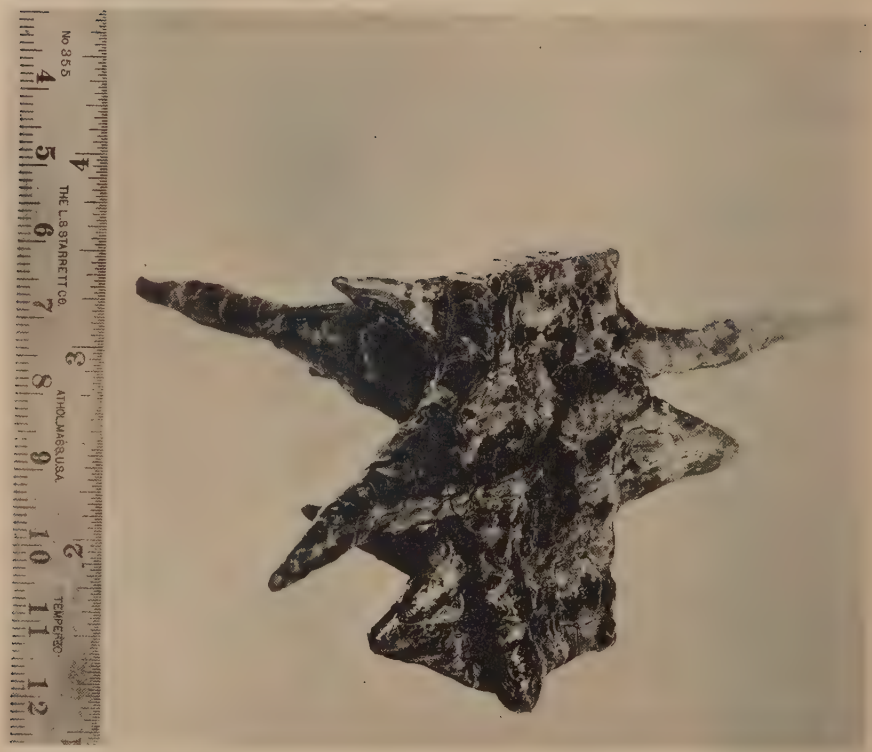

Figure 3

Fig. 3 Reconstruction in beeswax of anterior horn cell from the dorsolateral group of the fifth cervical segment. Reconstruction from camera-lucida drawings of successive serial transverse sections. The magnification is 600, indicating that the cell is 100 μ long. The considerably smaller dextrosinistral and dorsoventral diameters can be seen particularly at the upper surface of the cell. These diameters are, respectively: dextrosinistral, 53 μ, and dorsoventral, 48 μ (in the widest portion of the cell). The dendrites in the cephalocaudad axis, which is the vertical axis of the cell, as seen in the photograph, have not been indicated in the model, since, as the text indicates, they cannot be followed with certainty in successive serial transverse sections (fig. 2).

Fig. 4 Reconstruction in beeswax of anterior horn cell from the ventromesial group of the fifth cervical segment. Camera-lucida drawings from successive serial longitudinal sections cut in the dextrosinistral plane. The magnification is 600, indicating that the cell body is 92 μ long. The dextrosinistral diameter is the horizontal diameter in the photograph of the cell, while the cephalocaudad is the vertical diameter. The dextrosinistral diameter is 23 μ and the dorsoventral is 45 μ. The length of the upper long dendrite measured from the cell body is

Figure 4

150 μ. The dorsal and ventral dendrites have not been indicated in the model, since, as the text indicates, they cannot be followed in successive serial sections for the same reason as indicated in figure 3.

dextrosinistral plane the cell can be traced but three sections (15 μ thick). The dorsal and ventral dendrites are not easily traced (fig. 4).

The dextrosinistral diameter of the cells when photographed in longitudinal sections varies from 9 to 30 μ. This depends upon the plane through which the cell is cut. If this plane passes through its middle, the width is great; if to one side, much less. This is easily demonstrated by drawings and wax models of successive serial sections either longitudinal or transverse.

The observations here made are based upon examination of the cervical, thoracic, and lumbar regions of seven normal human spinal cords.

The technical difficulties in obtaining longitudinal sections are considerable. It is not easy to embed a piece of spinal cord so that it will be precisely straight, without minute bends and kinks. Further, the cord must be cut in a precisely longitudinal plane or else the cells appear spherical. The farther the plane is off from the longitudinal, the rounder the appearance of the majority of cells. Celloidin was used for embedding. Paraffin cannot be used on account of shrinkage of the cells.

The literature on the subject is meager. Cajal(1) states that the motor cells are from 60 to 70 μ in size and even larger, that they are multipolar and may be spherical, triangular, fusiform, or pyriform. He makes no comment upon any difference between the longitudinal and the other two diameters. His illustrations do not show the pictures of figures 1 and 2. The descriptions in Quain's Anatomy(2), Tilney and Riley(3) and Cunningham's Anatomy(4) are taken from Cajal. The texts of v. Bechterew(5), Spielmeyer (6), Van Gehuchten(7), and Winkler(8) give no further information than Cajal, either in their text or in their illustrations.

The significance of these findings in regard to the oblong form of the larger motor cells of the anterior horns is not entirely clear. Two factors may be considered. One is

purely physical—an accommodation of the cell form to the distinctly cylindrical form of the spinal cord. The other is neurobiotactic. The cells may be connected by their dendrites to many afferent fibers spread out in longitudinal fashion in successive segments, and thus the dendrites reach longitudinally to as great an extent as possible. We believe this latter theory the more probable of the two.

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THE NUMBER OF CORPORA LUTEA IN SUCCESSIVE MOUSE PREGNANCIES

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FOUR FIGURES

While litter size is the most accessible measure of reproduction in rodents, it is unreliable as an index of ovarian activity, or fecundity, as it is always subject to modification by concealed prenatal mortality. Investigations upon numerous mammals in which counts of ova or corpora lutea have been compared with average litter size (for references see Corner, '23) indicate that the average prenatal mortality is very high, ranging from 30 per cent to 40 per cent and in some species even higher. With prenatal mortality so high and subject to influences quite independent of those determining the number of ova, it is clear that a study of ovarian activity must be made from the ova or the ovaries themselves. A method of counting directly the mature ova formed by a mammal in successive ovulations has not been discovered. The nearest approaches to this have been made by such studies as that of Corner ('23), who made microscopic counts of the ova collected from the uteri of sows, and of Long and Evans ('22), who have made microscopic counts of ova in sectioned Fallopian tubes of rats. Such studies do not consider the age of the animals, and obviously they could not give data upon successive ovulations. Allen ('22) gives counts of as many as three different sets of bodies in the same ovary, follicles and corpora lutea of different ages, but again the age of the animals is not considered. Hammond ('14) does con-

sider age by distinguishing between 'young' and 'old' sows. He found an average of 14.3 corpora lutea (range, 11 to 19) in young sows and 19.7 corpora lutea (range, 13 to 19) in old sows. From this he concluded that the lower fertility of young sows is due to the formation of a smaller number of ova.

While the corpora lutea formed may not be identical with the number of ova liberated, it gives a direct measure of the activity of the ovary. Even if a corpus may be formed from a follicle without the extrusion of the ovum, as Long and Evans have found, the reliability of the corpora as criteria of ovarian activity is not seriously impaired, for in such cases the ovum must have reached its final stage of development in order to initiate the formation of the corpus, and, as far as the ovary itself is concerned, is a mature egg that should be included in the count. Since the corpora lutea are the only possible index of fecundity in the living animal, the best practical definition of a clutch of mammalian ova may be the number of ova that develop far enough for the production of corpora lutea. From a technical standpoint, the ease of observing and counting the corpora lutea of pregnancy in mice by means of a surgical operation makes it possible to obtain fairly large numbers of counts. This paper is based upon 5968 corpora lutea in 611 ovulations of 123 mice.

METHODS

The mice used came from five lines that had been inbred, brother and sister, for several generations, in most cases for five or more. The females were weaned at four weeks and, in units of not less than four litter mates, were given a closely related male (father or older brother) whose fertility was already demonstrated. Very close watch was kept for pregnancies. By palpation, pregnancy was usually determined from six to nine days before birth. From this it will appear that if any pregnancy was not observed, it probably was terminated by death and resorption of all the embryos in less than two weeks. In other words, any theo-

retical error in the results due to the failure to observe a pregnancy must be very slight. As long as a mouse was under observation it was observed upon at each pregnancy[1] and each one given an individual box until the birth of the litter. Within twenty-four hours of this time the young were removed and the female remated. In so many cases the interval between births was twenty-one days that during the period covered for each female, the number of pregnancies must have approached the number of ovulations. Half of each unit of females was treated with alcohol. This divides the data into two random groups. Each group of data alone would lead to the conclusions herein reached, so the data from the treated mice are included, but only to increase the numbers, as this paper does not deal with the subject of alcohol.

The surgical technique employed in examining the corpora lutea of pregnancy is essentially the same as that previously described (MacDowell, '24). The principal change has been the use of a chlorazine bath for the ligatures, in order to prevent the occasional formation of a stitch abscess between the skin and the body muscles.

EVALUATION OF DATA

In the third week of pregnancy the corpora lutea corresponding to the litter in utero are differentiated from all others as brilliant red bodies protruding from the surface of the otherwise pale cream ovary. The membranous capsule that completely surrounds the whole organ is perfectly transparent and frequently distended by a transparent fluid that magnifies the surface of the ovary. In the early operations the chance of overlooking a corpus is almost nil. The one source of possible error lies in misinterpreting two corpora that lie closely appressed. In such cases, however, there are usually structural indications of this doubling which stand

[1] Two litters were born before the observation of the corpora lutea, through an accidental delay in the operation. At birth, the corpora lutea so completely lose their brilliant coloring that postparturitional counts are not reliable.

out clearly under the binocular always used in making the count. Furthermore, the variability in the diameter of corpora in an ovary is sufficiently limited for the identification of two closely united corpora by the size alone.

Another source of possible inaccuracy is in the later counts after the increase in ovarian fat and the formation of adhesions at the wounds have limited the freedom of exposing all sides of the ovary. In many cases the adhesions were separated, and often the fat could be dissected from the surface of the capsule. If this could not be accomplished satisfactorily, so that all sides of the ovary were viewed, the count was obtained by cutting the capsule and slipping the ovary entirely out. While the counts can be considered strictly accurate only as minima, overlooking a corpus must be a rare occurrence even in late operations, for a close check has been obtained by removing the capsule in the last operation after an acceptable count has been made by the usual method. A general check on the reliability of these counts can be obtained from comparisons with counts made from sectional material. Long and Mark ('11) report that the number of eggs maturing at each ovulation in the mouse is nearly seven; Parkes ('23) gives figures that indicate an average of 7.1 corpora lutea per ovulation. Allen ('22) found 9.2 corpora lutea or large follicles per oestrus. For comparison with these counts previously reported averages obtained by the present method (MacDowell, '24 a and b) were 9.4 (136 counts) and 9.9 (115 counts). The present data average 9.8 (611 counts). However the differences in these various averages may be explained, they cannot be explained by supposing that corpora are overlooked in the operative method.

RESULTS

Counts of corpora lutea classified by order of litter (parity):

Figure 1 presents a summary curve based on the averages of all the data together. The curve rises with the number

of the litter, but between the first and third litters there is as great a rise as is found in the rest of the curve from the third to the tenth litter. As indicated on the figure, the number of cases falls with each litter after the second. In

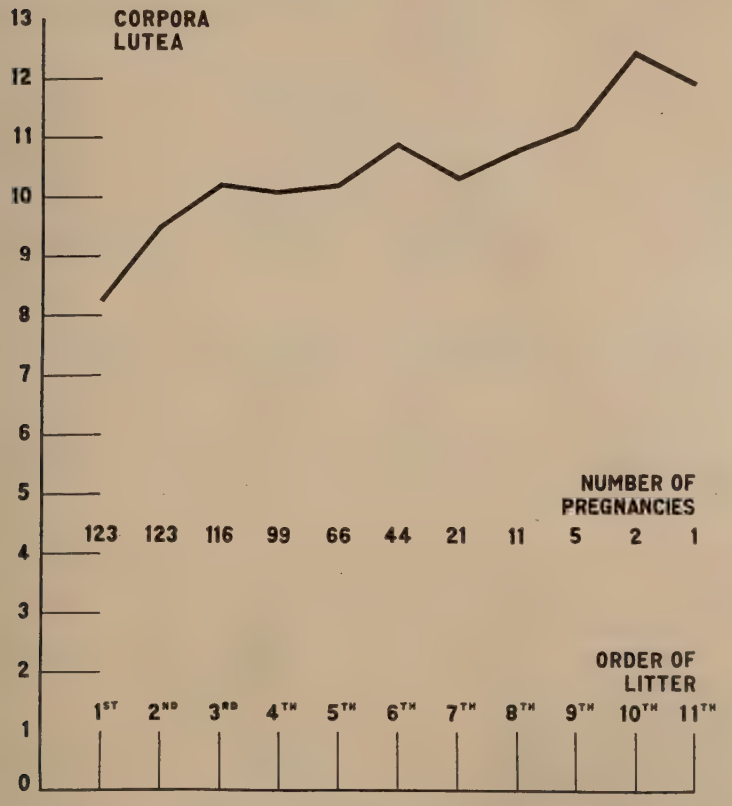

Fig. 1 Average number of corpora lutea classified by order of the litter; all data.

order to present results free from the possibility of a selective effect in this falling off in numbers, the averages for mice having five counts are given separately in figure 2 (all strains). In this figure the counts from the normal mice

and those treated with alcohol are given separately. Although this pair of curves is based on only thirty-nine and twenty-five mice, each one gives the same general results as the first curve—a rapid rise to the third litter followed by a marked flattening, but no actual decline. The following pairs of curves in figure 2 give the data subdivided according to the strain.

All the original data, unmodified by any classification or arithmetic are presented graphically in figure 3, in which each dot represents a count with the number of corpora lutea given on the scale at the left, for a litter whose order is given by the scale at the bottom. The coefficients of correlation (table 1) give mathematical expression to the relationship

TABLE 1

Coefficients of correlation between number of corpora lutea and order of litter. All fathers normal; mothers in second line treated with alcohol

	ALL LITTERS	FIRST TO THIRD LITTERS	LATER LITTERS
Normal females	$+0.359 \pm 0.033$	$+0.389 \pm 0.042$	$+0.165 \pm 0.055$
Treated females	$+0.369 \pm 0.034$	$+0.428 \pm 0.041$	$+0.176 \pm 0.063$

shown in the figure. Figure 3 includes all the mice, but the correlation coefficients are given separately for the normal and the treated mice.

Considering all litters, the coefficients of $+ 0.36 \pm 0.03$ and $+ 0.37 \pm 0.03$ indicate a strong degree of relationship. However, the averages show that the correlation cannot be actually linear; this raises a question as to the significance of these coefficients. A closer approach to linearity is obtained if the data for first, second, and third litters are taken separately from the remainder. In this case the coefficients rise to $+ 0.39 \pm 0.04$ and $+ 0.43 \pm 0.04$; for the later litters alone the coefficients are considerably lowered, $+ 0.17 \pm 0.05$ and $+ 0.18 \pm 0.06$. These low coefficients of correlation for the late litters give, from a different arithmetical angle, the same conclusions as the simple averages, i.e., after the third

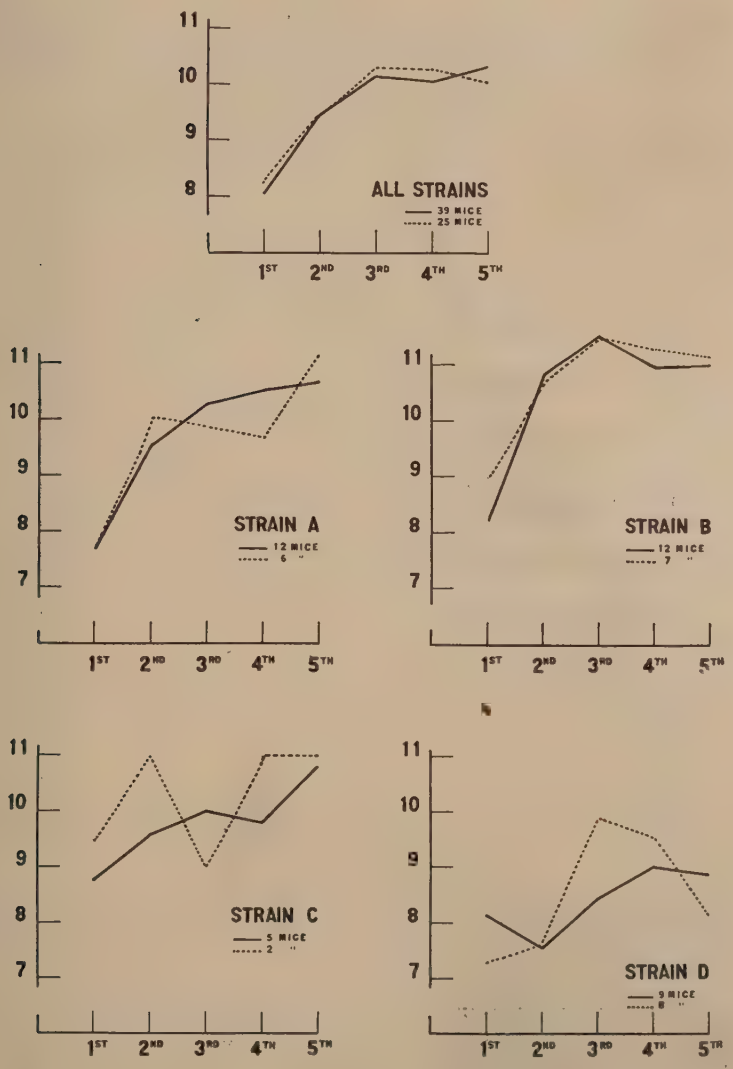

Fig. 2 Average number of corpora lutea classified by order of litter; only mice with five counts included; data subdivided according to strain; solid line, normal females; dotted line, females treated with alcohol.

litter there is a continued, but greatly reduced, tendency for the number of corpora lutea to increase with the number of the litter.

A question of interest is whether the rise in the corpora lutea counts is due to age alone or whether the suspension of

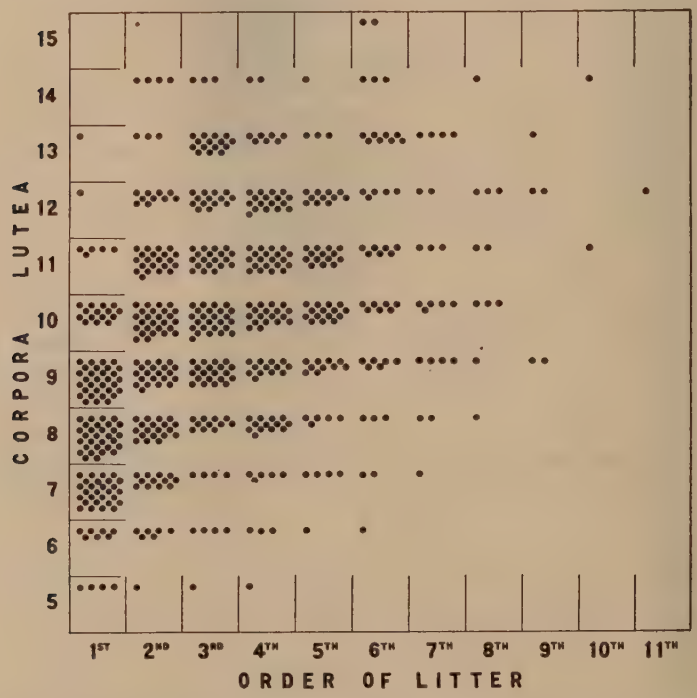

Fig. 3 Original data, unclassified, on the relation between number of corpora lutea and order of the litter; each dot represents a count of corpora lutea and the order of the corresponding litter.

ovulation during pregnancy and the processes of gestation themselves are factors that influence the number of corpora. If the averages rose more regularly when classified by age than by the order of the litter, we would be led to believe that age was the main, if not sole, factor. But the two classifications do not give consistently different results and the cor-

relation coefficients based on the age of the mother are so nearly like those given for parity as to show nothing more than a close correlation between parity and age of mother.

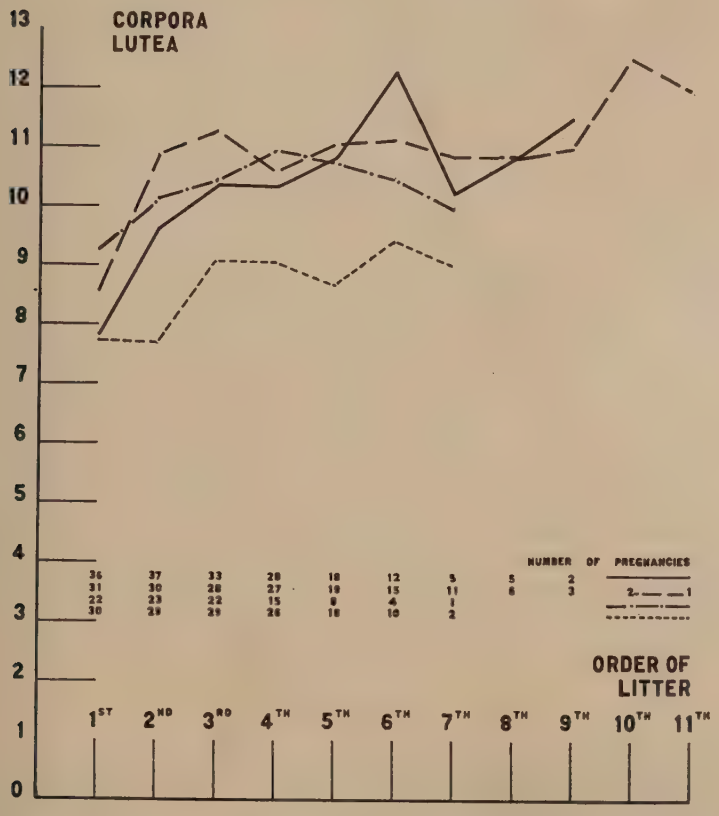

Fig. 4 Comparison of average counts of corpora lutea in four strains. All data. Solid line, strain A; broken line, strain B; dot-and-dash line, strain C; dotted line, strain D.

Influence of strain difference on the number of corpora lutea: The curves in figure 4 give the averages of corpora lutea for the four principal strains separately. Each curve seems to have certain characteristics of its own. *A* begins

low and rises till it overtops the others; *B* begins the highest and shows least rise; *C* makes its main gain in the second litter and shows little change afterward. That these three strains differ genetically in the production of corpora lutea would be an unsafe conclusion, but in the case of the fourth, strain *D*, the averages are so consistently and widely separated after the first litter and the course of the curve is so different from the other three, that it seems necessary to suppose some biological difference is involved. Since the environmental factors, the management of the breeding and technical methods, have been the same for all strains, it is concluded that the difference in the production of corpora lutea between this and the other strains is genetic.

Allen ('22) suggested that a supervening pregnancy might reduce the number of ova produced at the oestrus following parturition, and so account for the difference between his average for the number of corpora lutea and that given by Long and Mark. Since a high proportion of the counts averaged above were from the ovulations immediately following parturition and as the numbers in successive ovulations rise rather than fall, this suggestion does not seem to be supported. Genetic differences between the strains used by different authors, and age differences of the females would seem to be more plausible explanations of the different averages for the number of the corpora lutea.

CONCLUSION

1. The number of corpora lutea produced by the ovaries of a mouse is correlated with the age of the mouse in terms of the number of litters previously thrown. Judged by the corpora lutea of pregnancy, there is a continuous increase in the activity of the ovary of a mouse during the early part of its breeding period (first and third litters); after this the further increase is sharply reduced, but as far as the ninth to eleventh litter there appears no tendency for the number to decline.

2. The number of corpora lutea is influenced by genetic characteristics of different strains.

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DATA ON THE PRIMARY SEX RATIO IN THE MOUSE

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The literature upon mammalian reproduction appears to include no direct data upon sex ratios at the time of conception—that is, primary sex ratios. Indeed, such evidence has been considered obviously unobtainable. The nearest approaches have been made by projecting back to the beginning of pregnancy the trend of sex ratios obtained from abortions at different ages, stillbirths, and living births. This method would yield reliable results if the differential viability of the two sexes remained the same or favored the same sex throughout embryonic life. But this has never been demonstrated to be a fact, and it cannot be demonstrated so long as any considerable part of the prenatal deaths are not sexed. In domestic and experimental animals the usual prenatal mortality is high and little is known of sex ratios before the later part of pregnancy. In the white rat King ('21) found less than 2 per cent stillbirths in over 4000 litters and too few abortions to be considered. Studies in which the number of corpora lutea and the average litter size have been compared—in the pig (Corner, '23; Hammond, '21), the rat (Long and Evans, '22), the ferret (Robinson, '21), the rabbit (Biedl, Peters and Hofstätter, '21, and Hammond, '21), the mouse (MacDowell, '24)—have revealed that the normal amount of prenatal mortality ranges from 30 per cent to 40 per cent and even higher in certain cases. It is clear from this that the influence of the main part of prenatal mortality upon the sex ratio is an unknown quantity in these animals. Even in human records, which have provided the most extensive

series of data on sex ratios in abortions, the total prenatal mortality is unknown. The mortality in the first month is completely hidden, and in all probability it is very high. This can be inferred from the fact that the abortions for the second and third months include half of all the cases. Franz ('98) gives 75.1 per cent; Auerbach ('12) 47.2 per cent. And this is true in spite of the fact that the data for these months are admittedly the most incomplete. It seems safe to conclude that an indefinitely large part of the prenatal mortality that actually occurs in man is unrepresented in the records. Furthermore, as long as the influence of this unrecorded early mortality upon the sex ratio is only a matter of surmise, all statements about the primary sex ratio are unreliable; even the otherwise careful statement of Schultz ('18, p. 265) must lose its certainty: " . . . . it may be stated with certainty that more males (probably not more than 10%) are conceived . . . . ". Until all the prenatal mortality is accounted for, it will be impossible to determine primary sex ratios; a high proportion of males in abortions and stillbirths is not necessarily due to a high primary sex ratio, nor to a general susceptibility or weakness of male foetuses. The same results would follow if the primary ratio were 1 : 1 and there occurred a selective elimination of female embryos in the masked prenatal mortality during the early stages of development. Without knowing the primary sex ratio, the interpretation of the subsequent ratios is a matter of opinion.

This paper presents data on the primary sex ratio of the mouse based on litters which have suffered no loss from prenatal mortality.

In order to distinguish litters that have suffered no loss—that is, complete litters—it would seem necessary to know the number of ova that started each litter. To count the ova and find the sex of the young each ovum produces is the most simple matter in the bird; in the mammal this still seems an impossibility. But to count corpora lutea in a mouse and sex the corresponding young at birth is very nearly the same thing, because a follicle upon discharge of its ovum

develops into a corpus luteum. The discrepancy lies in the possibility of a corpus being formed with an ovum retained or more than one ovum coming from a single follicle. (For literature on this subject see MacDowell and Lord, '25.)

The surgical technique for making the examination of the corpora lutea of the mouse in the last week of pregnancy has been described previously (MacDowell, '24), and evidence has been presented showing that this operation does not interfere with the normal birth of the litter nor affect the subsequent pregnancies of the female. At this time the corpora lutea corresponding to the young in utero are the only large bodies in the ovary; they protrude far out from its surface and their intense red makes them stand out in striking contrast to the pale cream color of the rest of the ovary. Under a binocular magnifier of 7 diameters these corpora lutea are counted with ease. By delicate manipulation the ovary can be turned about within its transparent membranous capsule to reveal the corpora on all sides. A further discussion of the reliability of these observations is given elsewhere (MacDowell and Lord, '25).

Counts of the corpora lutea of pregnancy have been made for about 1200 litters which were sexed at birth. All young were killed at once and a close check on the accuracy of the sexing was secured by frequent autopsies; in every case of any uncertainty as to the external signs, the gonads were examined directly. A transverse incision near the pubis will permit the testes to slip out; these are glistening bead-like organs that lie at the base of the distended bladder. The ovaries, lying close to the kidneys, are so small that only the coils of the Fallopian tubes and the thread-like horns of the uterus can be distinguished with the naked eye.

Among the litters for which counts of the corpora lutea were obtained, 106 were found in which each corpus luteum was represented by a mouse sexed at birth—that is, litters unmodified by prenatal mortality. These litters are divided into two sets (table 1): sixty-eight from normal parents and thirty-eight from matings in which the father was heavily

TABLE 1

Number of males and females in individual litters that suffered no prenatal loss; in third section of table, fathers were treated with alcohol

PARENTS NORMAL								FATHERS TREATED			
Mother's No.	Order of litter	♂♂	♀♀	Mother's No.	Order of litter	♂♂	♀♀	Mother's No.	Order of litter	♂♂	♀♀
14331	3	3	6	19222	1	1	2	16532	1	6	3
14331	6	5	4	19222	3	5	5	16642	1	5	3
14334	2	3	4	19224	1	6	3	16668	3	4	5
15787	1	3	3	19226	1	3	4	16806	1	3	4
15925	1	5	5	19264	2	6	4	16807	4	3	3
16017	4	1	7	19458	1	7	3	16808	1	4	2
16208	3	2	3	19459	4	5	5	16912	3	4	4
16302	1	4	3	19461	2	2	8	17054	1	4	4
16531	1	5	3	19546	1	4	3	17171	1	3	5
16642	2	5	3	19577	1	3	4	17173	1	2	7
16667	2	5	3	19698	3	2	4	17913	2	10	2
16668	1	4	3	19741	1	6	3	17914	5	5	5
16807	1	2	5	19902	2	2	5	17988	1	7	3
17053	1	5	4	19904	1	5	2	17989	4	3	5
17055	1	3	2	19904	5	3	5	17990	1	2	6
17055	3	2	4	19905	2	4	5	18572	1	5	5
17055	5	1	4	22495	1	6	3	18809	1	3	3
17091	1	4	2	22920	1	4	4	19090	1	3	5
17094	4	5	4	23056	1	1	6	19092	3	5	4
17171	2	4	4	23218	1	4	2	19096	2	2	5
17283	1	7	3	23322	2	4	4	19226	4	4	5
17915	1	4	4	23324	2	4	3	19264	1	5	4
17989	5	3	5	23329	2	4	3	19459	1	3	6
18059	2	4	6	23819	1	4	2	19461	1	5	5
18060	1	5	4	23820	2	6	4	19461	3	3	6
18106	3	4	6	23887	4	5	2	19785	2	6	4
18571	1	5	4	23892	2	4	3	19786	1	3	5
18575	1	2	5	24045	1	3	4	23055	1	5	2
18808	1	5	3	24282	1	1	6	23217	1	3	2
18911	2	5	4	24451	2	3	5	23298	1	4	3
19019	1	7	1	24483	1	2	4	23330	1	3	4
19022	2	3	3	25821	1	3	5	23331	2	3	2
19094	1	5	2	25829	1	2	5	23364	1	4	5
19195	1	3	3	25831	1	4	3	23820	3	2	7
								23891	2	6	1
								24558	1	4	2
								24694	1	5	3
								25829	2	4	4

treated with alcohol. As this paper does not deal with the effects of alcoholic treatment, the data from the experimental animals are given only as substantiation of the results from the normal parents. However, in passing, it will be noted that the close agreement of the sex ratios from these two sets of litters does not support the conclusion of Bluhm ('24) that alcohol treatment of male mice raises the sex ratio through a selective effect upon the sperm. In the sixty-eight litters from normal parents (table 2) were found 261 males and 262 females. This is as near a 1 : 1 ratio as is possible with an odd number of individuals. In the thirty-eight litters from treated fathers were found 155 males and 153 females, a deviation of 1.0. Since both sets of data are close to a

TABLE 2

Summary of males and females in complete litters

PARENTS	NO. OF LITTERS	♂	♀	DEVIATION FROM 1:1	DEVIATION P. E.
Normal	68	261	262	0.5 ± 7.7	0.06
Fathers alcoholic	38	155	153	1.0 ± 5.9	0.17
Total	106	416	415	$0.5 + 9.7$	0.05

1 : 1 ratio, they may be combined, giving 416 males and 415 females.

If the ratio of 108+ predicted for man by Jendrassik ('11) and Schultz ('18) had been found, this total would have been divided into 432 males and 399 females. Neither this calculated primary ratio, nor the still higher ratio of 125, proposed by Auerbach ('12) is supported by the present data from the mouse, but considerably larger numbers will be required to establish this point beyond question. Further experiments are being undertaken.

CONCLUSION

For 106 litters of mice in which the number born was no less than the number of their corpora lutea, the primary sex ratio is found to be almost exactly 1 : 1.

June, 1925.

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VENOUS SYSTEM ASSOCIATED WITH THE LIVER OF A 6-MM. PIG EMBRYO

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SIX FIGURES

INTRODUCTION

This report is based chiefly upon a sagittal series of a 6-mm. pig embryo, first used by Professor Allen in the preparation of a pedagogical paper. The sections are 10 μ thick and are stained with carmine. Serial sections of other pig embryos of 3.5 to 12 mm. in length were examined to determine the constancy of structures.

Blotting-paper models of the liver region were constructed from the 6-mm. series, somewhat after the methods of Gage and Schaeffer. The model shown in the plates represents the blood vessels cast solid. The portal vein with its branches was removed before photographing the model. Photographs of a model of hepatic tissue and adjacent structures, used in tracing the course of some of the smaller vessels, are not shown.[1]

RELATION OF LARGER VESSELS TO SINUSOIDS

In this embryo the course of the main hepatic channels represents a stage midway between Bradley's nineteen-day-old and 8-mm. pig embryos. The development is further advanced than the 4.9-mm. human embryo of Ingalls, and although the similarity between the two embryos is close,

[1]A more detailed report, including several additional figures, is contained in a thesis presented to the Graduate Council of the University of Oregon, as part of the required work toward a Master of Arts degree, 1923.

there are certain marked differences. As seen in figure 1, the left umbilical vein is larger than the right and there is an anastomosis between the two veins within the septum transversum. The ductus venosus is well formed and the caudal portion of the left omphalomesenteric vein has been separated from the liver by the body cavity. Omphalomesenteric venous rings, about the intestinal tract, so prominent in earlier stages, have practically disappeared in this embryo. No large anastomosis between the left umbilical vein and the right omphalomesenteric vein is found, although it is a fairly constant structure in other embryos, as described by Bradley. Only the right mesenteric vein connects with

ABBREVIATIONS

a, aorta
als, portal anastomosis with left subcardinal vein
ars, portal anastomosis with right subcardinal vein
at, atrium
bc, body cavity
c, constriction between sinus reuniens and posterior superior sinusoid
db, dorsal branch of ventral division of left umbilical vein
dlu, dorsal division of left umbilical vein
dv, ductus venosus
lcc, left common cardinal vein
lcs, left central group of sinusoids
lls, large sinusoid in left lateral lobe
lh, left horn of sinus venosus
lhv, left hepatic vein
lom, left omphalomesenteric vein
lu, left umbilical vein
lvv, left vitelline vein
mc, median communication between left umbilical vein and sinus venosus
mn, mesonephros
mv, mesenteric vein
mvs, medioventral sinusoids
o, opening of right umbilical vein into portal vein
olu, extrahepatic course of left umbilical vein
oru, extrahepatic course of right umbilical vein
pc, postcardinal vein
ps, sinusoid from portal vein to ductus venosus
pv, portal vein
rcc, right common cardinal vein
rcs, right central sinusoids
rh, right horn of sinus venosus
rhv, right hepatic vein
rls, right lateral sinusoids
rom, right omphalomesenteric vein
rp, right posterior sinusoid
ru, right umbilical vein
rvv, right vitelline vein
sao, sino-auricular opening
sc, subcardinal vein
sn, vein ventral to mesenephros
sr, sinus reuniens
sv, sinus venosus
ua, umbilical anastomosis
uab, branch to medioventral sinusoids
vb, veins of Broman
vc, vena cava inferior
vlu, ventral division of left umbilical vein
vm, union of vitelline and mesenteric veins

the vitelline. Vestiges of the early, extrahepatic course of the left and right umbilical veins still remain (fig. 1, *oru* and *olu*).

The hepatic sinusoids vary from mere capillaries, 10 μ in diameter, to large spaces such as the right posterior sinusoid (fig. 1, *rp*) which has a vertical diameter of 160 μ. Five sinusoidal groups are apparent in the model: one in each lateral lobe, three in the central lobe. The right and left lateral groups of sinusoids (fig. 3, *rls* and *lls*) are in relation

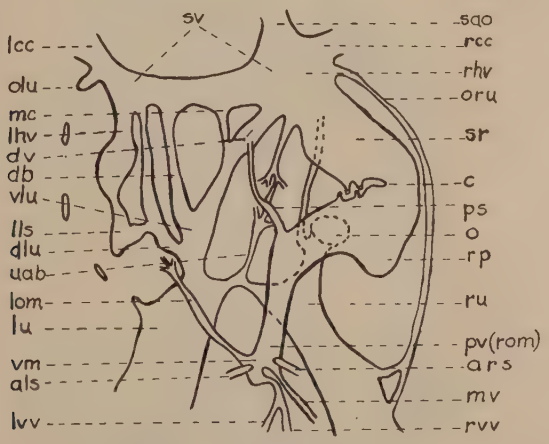

Fig. 1 Sketch of chief venous channels of liver, 6-mm. pig embryo.

to the right and left omphalomesenteric veins; the right and left central groups (fig. 2, *rcs* and *lcs*) to the two umbilical veins, and the fifth, or medioventral group (fig. 2, *mvs*) to a vessel (*uab*) running from the anastomosis between the two umbilical veins. A few capillaries in the septum transversum communicate with sinusoids of the left central group. These may later become transformed into hepatic sinusoids, as suggested by Mollier, Schulte, and Bremer.

Left umbilical vein. A slender mesenchymal band, covered by endothelium, extends dorsomediad and slightly cephalad across the lumen of the left umbilical vein near its entrance

into the septum transversum. This band does not appear in the figures. There is no corresponding band in the right vein, nor in the umbilical veins of other embryos studied. Entering the septum transversum, the left umbilical vein becomes abruptly reduced in caliber, then turning mediad, it anastomoses with the right (figs. 1, 2, 3). From this anastomosis arises a small vessel (*uab*) which supplies the medioventral group of sinusoids and ends in the ductus venosus. The medioventral sinuoids (fig. 2, *mvs*) lie ventral and cephalic to the gall bladder; they communicate with the right central group, of which they may be a part, and with the right posterior sinusoid.

Within the liver, the left umbilical vein (fig. 1, *lu*) separates into its dorsal and ventral divisions. Assuming a course dorsocephalad, the dorsal division (fig. 1, *dlu*) sends a short, wide branch to a prominent sinusoid (fig. 1, *lls*) in the left lateral lobe, communicates freely with the capillary sinusoids of the left lateral group, and continues cephalad as a small channel which terminates in the sinus venosus. The prominent sinusoid in the left lateral lobe, designated as the left lateral sinusoid (fig. 1, *lls*), receives the branch from the left umbilical vein near its blind caudal end. This sinusoid extends the length of the liver, communicating with the smaller sinusoids of the left lateral and left central lobes, and ends as the left hepatic vein (*lhv*) in the sinus venosus. Bradley suggests that the left lateral sinusoid may be a remnant of the left omphalomesenteric vein. The ventral division of the left umbilical vein (*vlu*), like the dorsal, is intimately connected with the hepatic sinusoids. It divides into two main branches. A small dorsolateral branch (fig. 1, *db*), the chief supply for the left central group of sinusoids (fig. 2, *lcs*), continues cephalad to end in the sinus venosus. The left central group is composed of freely anastomosing capillary sinusoids, lying largely to the right of the left umbilical vein. The major branch of the ventral division (fig. 5, *vlu*) may represent that portion which is to lie in the falciform ligament. This branch communicates with the sinus venosus in the median

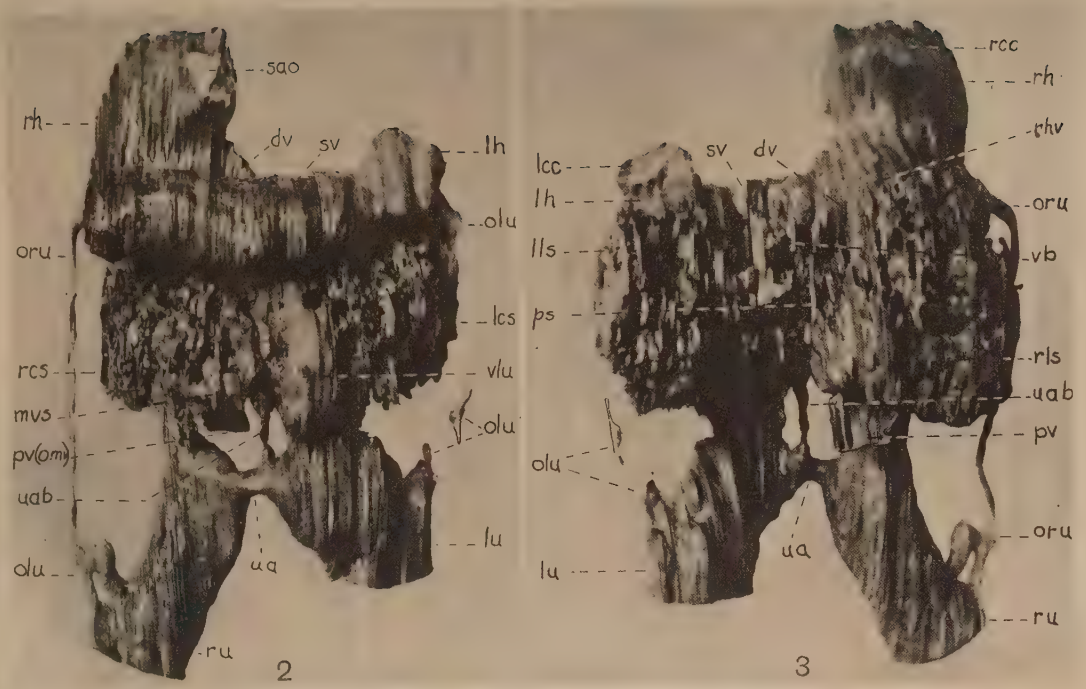

Fig. 2 Photograph. Model of liver sinusoids and veins. Ventral view. 6-mm. pig embryo.
Fig. 3 Photograph. Model of liver sinusoids and veins. Dorsal view. 6-mm. pig embryo.

plane (fig. 1, *mc*), then turns to the right and dorsally, forming the ductus venosus (*dv*). In its course the ductus venosus receives blood from the medioventral sinusoids and from the portal vein through other small sinusoids (fig. 1, *ps*).

The figures show the diameter of the right umbilical vein diminishing abruptly within the septum transversum. Beyond the anastomosis with the left umbilical vein, the right umbilical vein gives off a slender branch to the medioventral sinusoids. Upon entering the liver, the right umbilical vein enlarges and sends a short, wide branch dorsad to join the portal vein (right omphalomesenteric vein). Then, as a narrow sinusoid, it passes through the liver, supplying the right central lobe and ending in the right hepatic vein. Capillary sinusoids surrounding the right umbilical vein comprise the right central group (fig. 4, *rcs*); the body cavity partly separates the right central from the right lateral group.

Portal system. The paired vitelline veins unite in the umbilical stalk ventral to, and to the right of, the intestine. At the caudal extremity of the dorsal pancreas, the common vitelline vein expands into a large blood channel (fig. 1, *vm*) which has developed from the dorsal plexus between the caudal and cephalic omphalomesenteric rings of earlier stages. This channel receives the right mesenteric vein (fig. 1, *mv*) and anastomotic branches from the right and left subcardinal veins (*ars* and *als*). Allen mentions the anastomosis between the subcardinal and the portal system. This anastomosis is not an anomaly, for it is present in pig embryos of 6, 7, and 8 mm. It is clear that the right limb of the caudal omphalomesenteric ring has disappeared, while the left limb of the cephalic ring persists as a mere plexus of veins (fig. 1, *lom*) to the left of the dorsal pancreas. From the point of anastomosis with the right subcardinal vein, the right omphalomesenteric vein, or portal vein, continues cephalad as a vessel of 90 to 100 μ in diameter. It turns sharply to the right within the liver to join with the right umbilical vein in forming the right lateral sinusoid (fig. 1, *rp* and *sr*) which His interprets as a portion of the right omphalo-

mesenteric vein which has not broken up into smaller sinusoids, or which has been reopened. The right lateral sinusoid is constricted (fig. 1, *c*) into two parts, the right posterior sinusoid (*rp*) and the sinus reuniens (*sr*; vena hepatica revehens of Hochstetter; sinus S of Allen) which ends in the right hepatic vein.

Small sinusoids ventral to the stomach connect the right and left lateral lobes. These sinusoids, together with the cephalic portion of the ductus venosus, seem to be remnants of the cranial ring of the omphalomesenteric veins described by Hochstetter, Ingalls, and others. Figure 4 shows the veins of Broman (*vb*) to be intimately associated with these structures.

Sinus venosus. The transverse trunk (figs. 1 and 2, *sv*) of the sinus venosus (saccus sinus reuniens of His) is prominent between the heart and liver. It receives blood from the cardinals and from the dorsal division of the left umbilical vein. The greater portion of the blood passing through the liver, coming from the right umbilical vein, portal system, and ventral division of the left umbilical vein, pours into the right horn of the sinus venosus. Figure 2 reveals the sino-atrial opening (*sao*) in the ventral part of the right horn.

OTHER VESSELS

Since certain other veins, not so intimately associated with the liver, occur in the models, a brief description of them may be of interest. The inferior vena cava is not completely formed at this stage. It is a minute vessel which can be traced cephalad in the mesentery from the right subcardinal vein almost to the region of the veins of Broman. Paired veins (figs. 4, 5 and 6, *sn*), hitherto not mentioned in the literature, arise from the ventral wall of the posterior cardinal veins, immediately cephalad of the mesonephros. Each vein extends caudally, ventral to the Wolffian body, from which arise its tributaries. The paired veins are not to be confused with the subcardinals, which are medial to the Wolffian bodies in the 6-mm. stage. Figure 6 sketches roughly the development of these veins, now being investigated in more detail by Prof. C. F. W. McClure.

SUMMARY

As a result of a study of models of the liver region of a 6-mm. pig embryo, the following points were noted.

1. The sinusoids within the liver are divisible into five groups: left and right lateral, left and right central, and medioventral.

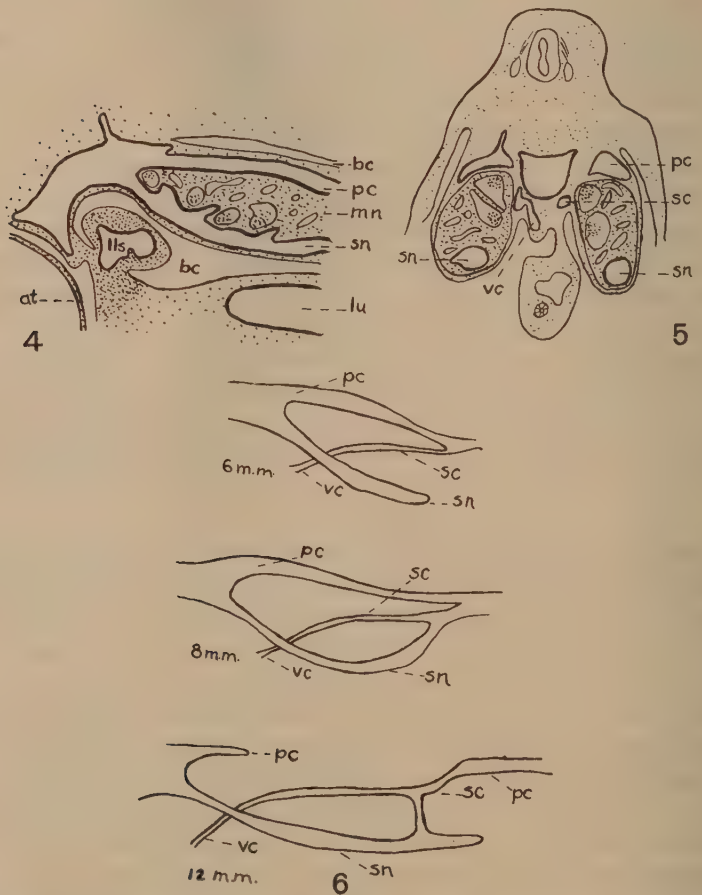

Fig. 4 Camera-lucida drawing. Sagittal section through left mesonephros. 6-mm. pig embryo.

Fig. 5 Camera-lucida drawing. Transverse section. 6.5-mm. pig embryo.

Fig. 6 Sketch of development of branch of postcardinal vein running ventral to mesonephros.

2. The left umbilical vein anastomoses with the right upon entering the septum transversum.

3. Within the liver the left umbilical vein breaks up into a dorsal division and a ventral division; the dorsal supplies the left lateral lobe through its communication with a large left lateral sinusoid, the ventral division supplies the left central lobe and forms the ductus venosus.

4. The extrahepatic course of the original left umbilical vein has almost disappeared; there is, however, an unbroken channel to the right of the liver marking the path of the original right umbilical vein.

5. The right umbilical vein enters the liver and joins the portal vein in forming a large sinusoid which occupies most of the right lateral lobe of the liver. This large sinusoid unites with the ductus venosus to form the right hepatic vein.

6. The portal system receives communicating branches from both the subcardinal veins.

7. It is the right horn of the sinus venosus which receives most of the blood from the liver.

8. At this stage the vena cava has not tapped the hepatic sinusoids.

9. A hitherto unreported branch of the postcardinal vein, ventral to the mesonephros, has been briefly described.

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QUANTITATIVE STUDIES OF THE TESTICLE

II. PATTERN AND TOTAL TUBULE LENGTH IN THE TESTICLES OF CERTAIN COMMON MAMMALS

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INTRODUCTION

Studies of the morphology of the testicle have been largely qualitative in character. Quantitative data, however, exist with reference to gross weight, tubule diameter, tubule length, and cell counts. As examples may be mentioned the work of Bouin and Ancel, Hatai, Jackson, Massaglia, Latimer, and Bessesen and Carlson, who have recorded gross weights of the testicles of various animals. Siperstein, in addition to gross weights, also gives the diameter of the seminiferous tubules and diameter of nuclei of interstitial cells. Moore, in a later paper on experimental cryptorchidism, gives gross weights and tubule diameters. Watson records seasonal changes in testicular diameter in the green finch. Riddle offers data on size and length relations in the right and left testes of the pigeon. Rasmussen, in his study of the seasonal changes in the marmot, employed cell counts of interstitial cells and records percentage weights of the testicles. Lengths of individual tubules were determined in the mouse, rabbit, and dog by Curtis. Huber and Curtis, writing on the seminiferous tubules in mammals, mention in an incidental way some quantitative data as to tubule length. Cunningham's Manual of Practical Anatomy, without citing the source of information, makes the statement that the human testicle "consists of four to six hundred fine hairlike tubes. . . ., each of which is about 60 cm. (two feet) long."

To our knowledge, no quantitative method has been applied to the measurement and comparison of total tubule length of the seminiferous tubules in a series of laboratory and domestic animals. In this paper such a method and the results of its application are set forth.

MATERIALS AND METHODS

Total tubule lengths of single normal testicles were computed for the following species: albino mouse, albino rat, Guinea pig, cat, Belgian hare, dog, man, pig, sheep, and bull. The testicles were separated from the epididymis and other extraneous tissue and were weighed in the fresh condition before fixation. In the case of certain representative testicles of the bull and pig, the tunica albuginea was stripped off and the mediastinum dissected out in the fresh condition and weighed separately from the rest of the testicle.

Bouin's fluid was used in general as the fixative, Zenker's fluid and 10 per cent formalin in a few cases. The testicles of the mouse, rat, Guinea pig, cat, hare, and dog were fixed for a brief interval entire and then sliced with a razor for further fixation. From the larger testicles blocks of tissue were removed with a razor and fixed. Both celloidin and paraffin were used for embedding, the former, to the greater extent, proving also the more satisfactory. The combination of stains most frequently used was hematoxylin-eosin.

The quantitative technique employed for determining total tubule length may best be presented by following successive steps in two typical examples.

Example 1. Bull testicle X 3. Fresh weight, 274 grams

By the steps previously outlined it was determined that the tunica albuginea and mediastinum weighed 15.5 grams (5.6 per cent of the total weight of this testicle). Hence tubules and interstitial tissue weighed 258.5 grams.

Several representative fields in sections of this testicle were projected with an Edinger drawing apparatus and the tubules drawn in outline. Then the relative amounts of tubules and interstitial tissue present in these drawings were measured in two ways: by

use of a polar planimeter and by the paper method already employed by Bascom in estimating the amounts of interstitial tissue in testicles of swine. In testicle X 3 the relative percentages of tubules and interstitial tissue were, respectively, 83.9 per cent and 16.1 per cent. Since, as already stated, tubules and interstitial tissue together weighed 258.5 grams, according to the above percentages the testicle was calculated to contain 216.88 grams of tubules. For all practical purposes the specific gravity of fresh tissue may be taken as 1.0, hence the 216.88 grams of tubules are assumed to be very nearly equivalent to a volume of 216.88 cc. or 216,880 cu.mm.

By means of a microscope with eyepiece micrometer, the average diameter of fifty seminiferous tubules was found to be 0.238 mm. Using the formula πr^2, the average cross-sectional area of the seminiferous tubules was calculated to be 0.0446 sq.mm. Dividing total tubule volume in cubic millimeters (216,880) by cross-sectional area in square millimeters (0.0446) gives the total tubule length of this testicle in millimeters (4,862,000). This sum is more compactly expressed as 4862 meters or as 4.862 kilometers.

Example 2. Guinea-pig testicle X 12 A. Fresh weight, 1.2423 grams

In this case the tunica albuginea and mediastinum could not conveniently be dissected away in the fresh condition. Representative transverse sections from different parts of the testicle were prepared. By means of the Edinger apparatus, a drawing was made from one of these sections and, with the aid of the planimeter, the percentage of mediastinum present was calculated (8.89 per cent). This was assumed to be the percentage of mediastinum in this testicle.

The volume of tunica albuginea was determined in a different way. Its thickness was measured with an eyepiece micrometer and found to average 0.031 mm. The Guinea-pig testicle approaches the spherical form rather closely. Accordingly, testicle X 12 A was assumed to be a sphere having a volume of 1.2423 cc., or 1242.3 cu.mm., the volume assumed to be equivalent to the weight of this testicle. The volume of a sphere equals $\frac{4}{3}\ \pi r^3$, hence a sphere of the above volume has a radius of 6.668 mm. The thickness of the tunica albuginea (0.031 mm.) subtracted from this radius gives 6.637 mm. as the radius of the smaller sphere, with a volume of 1223.6 cu.mm., representing tubules and interstitial tissue and mediastinum.

The percentage of mediastinum in this testicle, calculated as shown above, was 8.89 per cent, equivalent to 110.4 cu.mm. Hence the net volume of tubules and interstitial tissue becomes 1223.6 minus 110.4, or 1113.2 cu.mm. Of this volume 91.83 per cent, or 1022.25 cu.mm., was seminiferous tubules, determined as in example 1. Average tubule diameter was determined by micrometer measurement to be

0.244 mm. Employing the formula πr^2, the average cross-sectional area is 0.0467 sq.mm. Dividing volume of tubules (1022.25) by cross-sectional area (0.0467) therefore gives 21,889 mm., or 21.889 meters as the total tubule length in this testicle.

We believe these two examples sufficiently illustrate the methods by which the results to be set forth in this paper were obtained. Corrections have not been made and could not easily be made for certain minor errors, such as assuming the Guinea-pig testicle to be perfectly spherical, assuming the specific gravity of the testicle to be 1.0, or assuming that all elements of the testicle shrink equally under such technical treatment as we have employed, but we do not believe that they substantially affect our result.[1]

Thanks are due Mr. Herman Melton for technical assistance.

RESULTS

The numerical data obtained by the above methods are summarized in table 1.

DISCUSSION

In table 1 the column of gross weights of testicles shows great variation in weight in the different species—from 0.0556 gram in the mouse to 367 grams in a boar. In view of this great disparity in testicular weights, it is interesting to find that the diameters of the tubules in the above-mentioned testicles are, respectively, 0.191 mm. and 0.223 mm.—a differ-

[1]All calculations as to percentage composition are based on the planimeter method for the sake of uniformity and because we believe it to be more accurate than the paper method. We have also found it to be more rapid and less tedious. Results obtained by applying both methods to each of twenty-six testicles of animals of ten different species show an average discrepancy between the two sets of results of 2.48 per cent, figures obtained with the planimeter method being on the average slightly higher than those worked out by the paper method.

In the first paper of this series (Bascom, '25) no allowance was made for tunica albuginea and mediastinum in calculating the amounts of tubules and interstitial tissue in pig testicles. Relative percentages of tubules and interstitial tissue are not affected by this error, but absolute weights of these elements as given in that paper are about 8 per cent too large.

TABLE 1

SPECIES AND COLLECTION NUMBER	GROSS WEIGHT OF TESTIS IN GRAMS	WEIGHT OF TUBULES IN GRAMS	DIAMETER OF TUBULES IN MM.	CROSS-SECT. AREA OF TUBULES IN SQ. MM.	TOTAL TUBULE LENGTH IN METERS
Albino mouse					
M 1	0.0556	0.0477	0.191	0.0286	1.67
M 2	0.0616	0.0521	0.191	0.0286	1.82
M 3	0.0746	0.0641	0.2008	0.0316	2.03
Albino rat					
R 1	1.235	1.0584	0.2422	0.0458	23.1
R 2	1.13	0.9834	0.248	0.0484	20.3
R 3*	0.265	0.2248	0.1652	0.0213	10.55
R 5A	1.2497	1.0875	0.2494	0.0487	22.3
R 5B	1.2883	1.1414	0.2589	0.0524	21.7
R 11	1.0285	0.9031	0.2654	0.0553	16.3
Guinea pig					
X 11A	1.64	1.493	0.1979	0.0307	48.6
X 11B	1.67	1.5074	0.2084	0.0339	44.4
X 12A	1.2423	1.0222	0.244	0.0467	21.89
X 12B	1.1662	0.9794	0.2391	0.0448	21.8
X 13A	1.1665	1.031	0.2262	0.0402	25.6
X 13B	1.1846	1.0336	0.2315	0.042	24.6
X 20	1.9935	1.7160	0.2379	0.0443	38.7
Cat					
X 1A*	0.8	0.5726	0.1796	0.0251	22.8
X 1B*	0.94	0.6625	0.1806	0.0256	25.8
Belgian hare					
X 18	2.307	1.8556	0.1836	0.0264	70.2
X 19*	0.1975	0.1193	0.07	0.0037	32.2
Dog					
X 2A	6.0	5.0858	0.2165	0.0369	137.8
X 2B	6.5	5.618	0.2066	0.0335	167.7
Man					
X 10	12.21	6.577	0.1834	0.0263	249.7
Pig					
M 284A	218.0	125.29	0.225	0.0396	3164
M 283	367.0	246.18	0.223	0.039	6312
Sheep					
M 299*	15.0	10.2113	0.1127	0.0097	1052
M 137*	89.0	70.44	0.1475	0.0171	4119
M 170	166.0	129.48	0.2105	0.0345	3753
Bull					
X 3	274.0	216.88	0.2383	0.0446	4862
X 4	295.5	222.63	0.2383	0.0446	4991
X 5	203.0	147.46	0.1975	0.0306	4814
X 6	206.0	144.39	0.2357	0.0435	3319

* The asterisk indicates immature animals.

ence of only 14.3 per cent. Rat testicle R 11 had a greater tubule diameter (0.2654 mm.) than was found in any other testicle studied, not excepting even those of the boar, bull, or ram. The smallest average tubule diameter in the adult was found in the human testicle X 10 (0.1834 mm.). The maximum variation in tubule diameter in this series of testicles is then, in round numbers, 30 per cent. There is no direct relationship between tubule diameter and the size of the adult animal so far as our evidence goes.

TABLE 2

SPECIES	I. METERS OF TUBULES PER GRAM OR CUBIC CM. OF TUBULES	II. METERS OF TUBULES PER GRAM OF TOTAL TESTICULAR WEIGHT
Mouse	31.5–35.0	27.2–30.3
Rat (adult)	18.4–21.8	15.8–18.2
Rat (immature)	46.9	39.8
Guinea pig	21.4–24.8	17.6–21.9
Cat (not full grown)	39.0–39.9	27.4–28.5
Hare (adult)	37.8	30.4
Hare (immature)	267.0	163.0
Dog	27.5–29.8	22.9–25.8
Man	37.9	20.4
Pig	25.2–25.6	14.5–17.2
Sheep (M 170, adult)	28.9	22.6
Sheep (M 137, 6 mos.)	58.4	46.2
Sheep (M 299, 3 mos.)	103.0	70.0
Bull	22.4–32.6	16.1–23.7

The average cross-sectional area of the tubule in the rat testicle R 11 is 0.0553 sq.mm. and in the human testicle X 10 is 0.0263—in round numbers, a difference of 50 per cent.

The significance of tubule size may perhaps be more easily grasped when expressed as in table 2, column 1, in terms of the number of meters of tubules equivalent to 1 gram in weight or 1 cc. in volume.

Even after allowance is made for tunica albuginea and mediastinum, normal adult testicles vary in relative percentages of tubules and interstitial tissue. These variations in the species studied are summarized in table 3. In each case

the percentage of interstitial tissue is therefore equal to 100 minus the percentage of tubules given in the table.

Table 3 consequently indicates numerically the differences in pattern found in this series of normal adult testicles due to varying amounts of interstitial tissue.

Total tubule lengths for the adult animals studied are summarized in round numbers in table 4.

Incidental data exist from which total tubule length in the testicle of man and of the mouse may be calculated. Cunningham gives figures indicating a total tubule length in the human testicle of 800 to 1200 feet. Our calculation, involving a different method, gives 249.7 meters, or 819 feet.

TABLE 3

SPECIES	PERCENTAGE OF TUBULES IN TESTIS (EXCLUDING TUNICA ALBUGINEA AND MEDIASTINUM)
Guinea pig	89.8–97.0
Dog	92.2–94.2
Mouse	88.9–90.3
Hare	89.0
Rat	88.5–89.6
Cat	84.5–86.9
Sheep	84.6
Bull	76.4–83.9
Man	72.8
Pig	62.2–72.6

TABLE 4

SPECIES	TOTAL TUBULE LENGTH IN METERS
Mouse	2
Rat	20
Guinea pig	40
Cat (not full grown)	25
Belgian hare	70
Dog	150
Man	250
Pig	3000–6000
Sheep	4000
Bull	5000

Huber and Curtis state that the mouse testicle contains sixteen tubules. One tubule was found to be 13 cm. in length. These figures imply a total tubule length for the mouse testicle of about 2 meters. Our figure, independently calculated, is also about 2 meters. To this extent previous data secured by methods entirely different from ours confirm our computations.

At first glance the disparity in total tubule length between the larger and smaller mammals seems fairly incredible, but a little arithmetic will reveal that actually the smaller animals have a greater length of tubules per unit of body weight

than do the larger ones. For example, in round numbers, a mouse would have about 1 meter of tubules per 10 grams of body weight, while a bull would have approximately 1 meter of tubules per 100 grams of body weight. If the testicle made up the same percentage of body weight in the bull as it does in the mouse, the bull testicle would have, instead of 5000 meters of tubules, upwards of 300,000 meters. In this connection it is interesting to refer once more to table 1 and note that the mouse has a tubule diameter of almost 0.2 mm., while the bull has an average tubule diameter only 16 per cent greater (0.24 mm.). In other words, increased volume of germinal epithelium in larger testicles comes largely or wholly from increase in tubule length, not from increase in tubule diameter.

Certain immature animals are marked in table 1 with asterisks. Rat R 3 was thirty-nine days old and weighed 59.5 grams. His testicle weight was approximately 25 per cent that of a normal adult, but the total tubule length of his testicle was about 50 per cent that of the adult.

Rat R 2 was sixty-six days old and weighed 150 grams. His testicular weight and tubule length were almost those of the adult, although only in the earliest stages of sexual maturity.

Rat R 1 was 190 days old and weighed 304 grams, yet his testicular weight and total tubule length only slightly exceeded those of R 2, who was one-third his age and one-half his body weight.

Belgian hare X 19 was less than a third grown. His testicular weight was about one-eleventh and his tubule length two-fifths that of X 18, an adult of the same species.

M 299 was a lamb, perhaps three months old. Its testicular weight was about one-eleventh that of the adult, but its tubule length more than one-fourth that of the adult.

M 137 was a lamb about six months old. Its testicular weight was about half that of the adult, but its tubule length at least equal to that of adult M 170.

These comparisons of immature with mature testicles lead to the conclusion that total tubule length is greater in proportion to testicular weight in the young than in the adult testicle and that adult tubule length is probably attained in the growing animal a considerable time before adult testicular weight and adult tubule diameter have been reached.

The factors which determine testicular pattern are tubule diameter, relative amount of interstitial tissue, and amount of tunica albuginea and mediastinum. These factors are individually variable. The combined effect of their variability upon testicular pattern may be expressed numerically as in table 2, column II, in terms of the number of meters of tubules per gram of testicular weight. These data are obviously secured by dividing the total tubule length of given testicles in meters by their respective weights in grams. This column illustrates the following points: similarity in pattern in the adult testicles of certain species, differences in pattern in the adult testicles of other species, and changes in pattern during growth in a given species.

SUMMARY AND CONCLUSIONS

1. A quantitative method is described for determining the amounts of interstitial tissue and seminiferous tubules as well as the total length of these tubules. Testicles from animals of the following species were studied: albino mouse, albino rat, Guinea pig, cat, Belgian hare, dog, man, pig, sheep, and bull.

2. The gross weights of these testicles ranged from 0.0556 gram (albino mouse) to 367 grams (boar).

3. Adult tubule diameter had a maximum variation of only 0.1834 mm. (man) to 0.2654 mm. (albino rat).

4. Total tubule length in the adult varied from 1.67 meters (albino mouse) to 6312 meters (boar).

5. The small variation in tubule diameter in conjunction with the tremendous differences in total tubule length indicate that space for increased volume of germinal epithelium in the larger testicles is afforded largely and sometimes

wholly by increase in total tubule length, not by increase in tubule diameter.

6. In spite of the great total tubule length encountered in the larger species, the smaller animals, such as the mouse and rat, actually have a greater length of seminiferous tubules per unit of body weight.

7. The pattern of a given testicle depends upon the relative amounts of tunica albuginea, mediastinum, interstitial tissue, and tubules, and upon tubule diameter. The combination of these factors as they occur in a given testicle may be numerically expressed in terms of number of meters of tubules per gram of total testicular weight. This sum varies with the species studied as well as with the state of development of the individual (table 2, column II).

8. Total tubule length is greater in proportion to total testicular weight in the young than in the adult.

9. Adult tubule length probably is attained in the growing animal a considerable time before adult testicular weight and adult tubule diameter have been reached.

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BOOK REVIEW

GENERAL ZOOLOGY. By H. L. WIEMAN, Ph.D. (Chicago), Professor of Zoology in the University of Cincinnati. 1925: 286 pages, with glossary and index, 208 figures. McGraw-Hill, New York.

Doctor Wieman's little volume will be welcomed by the teacher of Zoology who wishes to put into the hands of his beginners a reliable statement of the essentials in this field, yet to reserve for himself the maximum freedom in presenting his own course.

This text can then be used as a substantial reminder of the main aspects of a course in Zoology, while the professor's lectures and the student's reading and laboratory notes are brought to the front as the most important illuminating features of his particular course. As Doctor Wieman points out, the laboratory work, field studies, and visits to the zoo or museum can be made to extend the course at will. In fact, this is required to attain a complete course, which may be thus made to vary from a general introduction to a more specific preparation for a professional course as medicine, agriculture, or a biological science.

Anyone familiar with our various conferences on the teaching of Zoology must recognize that the average zoologist will prefer this sort of text, though, at first, it may seem almost too condensed.

Doctor Wieman presents the essential facts of animal structure in a trustworthy comparative account, in terms of function. He pictures with clearness the principles involved in the study of animal forms and life.

There is restraint in discussion, as the author avoids attractive chances for digression and elaboration in the effort to leave these opportunities freely open.

Following the more general sections on cells, and adaptations, and brief chapters on special functional systems, on metabolism, etc., there is concise up-to-date consideration of the history of germ cells, ontogenesis, heredity, and evolution. The treatment here seems excellent as a basis of an understanding of topics often given more fully without such satisfactory clearness. It is definite, but not dogmatic. Each chapter is followed by a list of references, for the most part to more elaborate text-books readily available to the student, to encour-

age him to extend its contents by further reading. Important terminology is introduced throughout.

At the end of the book is added, in fifty pages, a general survey of the animal kingdom, giving the types, and furnishing brief characterizations with figures and many comments on peculiar features of structure, development, or habit. Much material is found here in a condensed form. Well-made illustrations supplement the text throughout wherever needed. There is also a glossary and index. The glossary is a specially valuable feature which might well be extended.

With this book as a class text, the reviewer feels that more students may, indeed, be led to venture further afield under the guidance of independent teachers. It will emphasize the primary importance of entrusting the teaching to zoologists of sufficient training and attainments to know how to meet the special needs of their students. Time and freedom will be left for special extensions of their work with the minimum requirements already furnished in this synoptic outline, which, however, contains all necessary explicit details.

An expectation that this text would encourage such tendencies to investigate further and independently could be no stronger recommendation for its use. More elaborate attempts where the aim is to say the last word as a finished story are probably less apt to exert just this influence. Such texts often appear to inhibit curiosity and initiative.

In any event, the teacher who adopts this outline for his class can depend on it as a reliable epitome of the subject, and teachers of 'premedical' students will find rather special attention to their needs.

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